R.J. Reynolds Tobacco Company

336-741-5000
Winston-Salem, N.C. 27102

November 25, 1998

C.W. Jameson, Ph.D.
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park NC 27709

Re: Ninth Report on Carcinogens, MD EC-14
Environmental Tobacco Smoke (ETS)

Dear Dr. Jameson:

R.J. Reynolds is submitting the enclosed materials in our efforts to assist the National Toxicology Program in its consideration of whether ETS warrants listing as a "known human carcinogen" or as being "reasonably anticipated to be a known human carcinogen."

You should have already received, along with a letter from Mary E. Ward dated Sept. 18, 1998, a copy of Judge Osteen's recent federal district court opinion that vacated the 1992 Environmental Protection Agency's Report on Environmental Tobacco Smoke.

Enclosed are 10 ETS exposure papers that have been published in peer-reviewed scientific journals. Also enclosed are two comments on drafts of the California EPA's report entitled "Health Effects of Environmental Tobacco Smoke.". We feel that these enclosures strongly support our belief that the scientific evidence is inadequate to classify ETS as a carcinogen or a suspected carcinogen.

Enclosed are:

1. Phillips K., *et al.*, "Assessment of Environmental Tobacco Smoke and Respirable Suspended Particle Exposures for Nonsmokers in Hong Kong Using Personal Monitoring," *Environment International*, Vol. **24**, No. 8, pp. 851-870 (1998).

2. Phillips K., *et al.*, "Assessment of Environmental Tobacco Smoke and Respirable Suspended Particle Exposures for Nonsmokers in Lisbon by Personal Monitoring," *Environment International*, Vol. **24**, No. 3, pp. 301-324 (1998).

3. Phillips K., *et al.*, "Assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Prague using personal monitoring," *Int. Arch Occup. Environ Health*, Vol. **71**, pp. 379-390 (1998).

4. Phillips K., *et al.*, "Assessment of air quality in Stockholm by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke," *Scand. J. Work Environ. Health*, Vol. **22** Suppl. 1, pp. 1-24 (1996).

5. Phillips K., *et al.*, "Assessment of Air Quality in Paris by Personal Monitoring of Nonsmokers for Respirable Suspended Particles and Environmental Tobacco Smoke," *Environment International*, Vol. **24**, No. 4, pp. 405-425 (1998).

6. Phillips K., *et al.*, "Assessment of Air Quality in Turin by Personal Monitoring of Nonsmokers for Respirable Suspended Particles and Environmental Tobacco Smoke," *Environment International*, Vol. **23**, No. 6, pp. 851-871 (1997).

7. Phillips K., *et al.*, "Assessment of Air Quality in Barcelona by Personal Monitoring of Nonsmokers for Respirable Suspended Particles and Environmental Tobacco Smoke," *Environment International*, Vol. **23**, No. 2, pp. 173-196 (1997).

8. Phillips K., *et al.*, "Assessment by Personal Monitoring of Respirable Suspended Particles and Environmental Tobacco Smoke Exposure for Non-Smokers in Sydney, Australia," *Indoor Built Environ.*, Vol. **7**, pp. 188-203 (1998).

9. Phillips K., *et al.*, "Measured exposures by personal monitoring for respirable suspended particles and environmental tobacco smoke of housewives and office workers resident in Bremen, Germany," *Int. Arch. Occup. Environ. Health*, Vol. **71**, pp. 201-212 (1998).

10. Jenkins R.A., *et al.*, "Exposure to Environmental Tobacco Smoke in Sixteen Cities in the United States as determined by Personal Breathing Zone Air Sampling," *Journal of Exposure Analysis and Environmental Epidemiology*, Vol. **6**, No. 4, pp. 473-502, (1996).

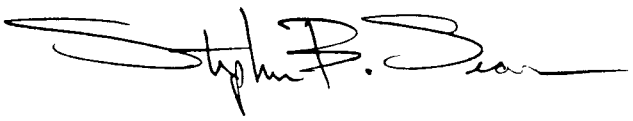
11. Rice SA, "Comments on Chapter 7: Carcinogenic Effects of Exposure to Environmental Tobacco Smoke; 7.2 ETS and Cancer Sites that are Associated with Active Smoking: Lung Cancer, submitted to California Environmental Protection Agency Office of Environmental Health Hazard, April 2, 1996.

12. "Comments of the R.J. Reynolds Tobacco Company on Health Effects of Exposure to Environmental Tobacco Smoke," submitted to California Environmental Protection Agency Office of Environmental Health Hazard, May 5, 1997.

I would also refer you to a publication by Phillips *et al.* examining ETS exposure in Basel, Switzerland. Unfortunately, I do not have a copy of that study, but I will forward one to you as soon as I can.

I hope these materials will be of assistance to the NTP as it considers the petition to list ETS.

Sincerely,



Stephen B. Sears, Ph.D.

Enclosures

COMMENTS ON

CHAPTER 7: *CARCINOGENIC EFFECTS OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE*

7.2 ETS AND CANCER SITES THAT ARE ASSOCIATED WITH ACTIVE SMOKING: LUNG CANCER

EXTERNAL REVIEW DRAFT: JANUARY, 1996

Submitted to

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Section

By

Susan A. Rice, Ph.D., D.A.B.T.

SUSAN A. RICE AND ASSOCIATES, INC.
SUNNYVALE, CALIFORNIA

Submitted April 2, 1996

1. EXECUTIVE SUMMARY

The U.S. EPA has, without regard to scientific method, made several basic--and incorrect--assumptions that fatally flaw Cal/EPA's ability to reach an unbiased, scientifically sound conclusion as to the relationship between environmental tobacco smoke (ETS) exposure and the risk of lung cancer. In accepting U.S. EPA's conclusion, Cal/EPA also accepts U.S. EPA's basic assumptions regarding ETS.

It is critical that Cal/EPA carefully assess all available and relevant toxicology data and make a thorough, unbiased, and scientifically-based assessment of the relationship or lack of relationship between exposure to ETS and lung cancer. Especially in light of the new information regarding the strength of the statistical associations in several of the epidemiology studies that Cal/EPA received during its workshop on March 25, 1996, it is imperative that the agency evaluate all available human and animal data relevant to the issue of ETS and lung cancer. In the case of ETS, the relevant toxicology data include a number of animal studies.

Pulmonary (bronchogenic) squamous cell carcinoma, the tumor type reported to be most closely associated with cigarette smoking in humans, has not been observed in either mice or hamsters exposed via inhalation to mainstream smoke, sidestream smoke, or ETS (Henry, 1986; IARC, 1986; Huber, 1989; Rodgman, 1992). Of all the inhalation studies performed, pulmonary squamous cell carcinoma has been observed in only one rat (F344) following exposure to cigarette smoke (Dalbey et al., 1980). The study was repeated two times (Heckman and Dalbey, 1982; Heckman and Lehman, 1985), but the initial observations were not confirmed.

The unique character of the complex mixture that is ETS makes extrapolation from mainstream smoke inappropriate. Contrary to the conclusions drawn by Cal/EPA based on

the epidemiology studies of ETS and the risk for lung cancer, the animal studies performed on aged and diluted sidestream smoke, the most appropriate surrogate for ETS, do not show and do not suggest an association between ETS exposure and lung cancer.

2. INTRODUCTION

Susan A. Rice and Associates, Inc. (SARA) is a consulting firm that specializes in the areas of toxicology and pharmacology. SARA has been asked to comment on Cal/EPA's external review draft by The R.J. Reynolds Tobacco Company. The comments provided herein are the result of review and analyses made by SARA, and the conclusions reached and the opinions expressed are those of SARA and do not necessarily reflect the conclusions or opinions of The R.J. Reynolds Tobacco Company. For the agency's information, the *curriculum vitae* and list of publications of Susan A. Rice, Ph.D., are included in Appendix I.

On March 25, 1996, the Office of Environmental Health Hazard Assessment (OEHHA) held a public workshop on environmental tobacco smoke (ETS) in order to elicit comments on its External Review Draft: *Carcinogenic Effects of Exposure to Environmental Tobacco Smoke: --Excerpt: ETS and Lung Cancer.* At that workshop, Richard A. Becker, Ph.D., Deputy Director for Scientific Affairs at OEHHA, stated that Cal/EPA intended to provide a scientifically-based assessment of ETS and the risk of lung Cancer. Furthermore, he stated that the agency was committed to including the best available information in its risk assessment.

The following information is provided to Cal/EPA to show that ETS is a unique, dilute, complex chemical mixture and to the point out some underlying assumptions that are currently a part of the review. The review draft document does not adequately scrutinize these assumptions. Specifically, it does not include a scientific assessment of the utility of

animal studies as an adjunct to the epidemiology studies, which have been used to evaluate the effects of ETS on the risk of lung cancer.

This commentary is not intended to be comprehensive. SARA recognizes that the limited discussions provide only an overview of selected aspects and do not adequately express the complexity of the subject matter. SARA acknowledges the level of energy and the depth of thought that Cal/EPA needs to invest to properly investigate the question of ETS and its potential to be a human pulmonary carcinogen. The basis for the assumption that ETS is a human pulmonary carcinogen needs to be critically evaluated, and science policy and health policy need to be clearly separated.

3. COMMENTS ON EXTERNAL DRAFT REVIEW

3.1. Draft Report Conclusions Based Primarily on Epidemiology Studies

Cal/EPA's conclusions presented in the external review draft inappropriately rest principally on the epidemiology studies related to ETS. The results of the epidemiology studies provide no consistent statistical evidence linking ETS exposure to the risk of lung cancer (e.g., Lee and Forey, 1995; Sugita et al., 1995; comments received at March 25, 1996, Cal/EPA workshop). Due to the very nature of epidemiology studies, there are many confounders that were not, and could not be, controlled even with the best of study designs.

Emphasis on the epidemiology studies completely ignores the fact that animal studies can augment the epidemiology studies, especially given the statistical weakness of the epidemiology studies, and advance the current scientific understanding of ETS, its potential to produce toxicity, and its relationship to mainstream smoke and to sidestream smoke. The draft review does not make one reference to or citation of an animal study.

3.2. Reliance on Analyses and Conclusions of U.S. EPA 1992 Report

Cal/EPA's draft report relies heavily on the analyses and conclusions of the U.S. EPA as contained in its 1992 report on ETS entitled *Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders* (U.S. EPA, 1992). Specifically, and without discussion or comment, Cal/EPA accepts U.S. EPA's conclusions that ETS is a human lung carcinogen and that there is compelling biologic plausibility of an effect of ETS exposure on the risk of lung cancer. Cal/EPA sought to support the conclusions of the U.S. EPA by stating that other earlier reports (IARC, 1986; NRC, 1986; U.S. DHHS, 1986) had reached similar conclusions.

In accepting the assumption that ETS is a human lung carcinogen, Cal/EPA has accepted the validity of U.S. EPA's evaluation of animal data. For several reasons that assumption is unwarranted and Cal/EPA's uncritical acceptance of that assumption demonstrates that the agency has not conducted an adequate review of the available data on ETS. First, the U.S. EPA examined only a selected subset of mainstream smoke and sidestream smoke exposure studies, and did not review all available relevant toxicology data. Second, U.S. EPA assumed that the observations made with mainstream smoke and sidestream smoke exposure studies were relevant to the evaluation of ETS and its relationship to lung cancer.

Propagating those assumptions will prevent Cal/EPA from conducting a scientifically valid analysis of the relationship, if any, between ETS and lung cancer. In order to claim a compelling biological plausibility, U.S. EPA had to assume that ETS is qualitatively the same as mainstream smoke, and that the observations made with mainstream and sidestream smoke studies are applicable to the evaluation of ETS exposure.

The U.S. EPA report (1992) did not consistently or adequately address the fact that ETS is a unique complex mixture that is different from both mainstream smoke and fresh

sidestream smoke (see sections 4.3 and 5.5 and Tables 1 and 2 in Appendix II). Because of inherent differences between the mixtures, observations made in mainstream smoke and sidestream smoke studies are not applicable to ETS. These differences include, for example, differences in particle sizes and particle retention in the lung, differences in the distribution of chemicals between the gas and particulate phases, and the extremely dilute nature of ETS compared with mainstream smoke and sidestream smoke (See Table 1). Dosimetrically, the difference between ETS exposure and cigarette smoking is immense. ETS is not dilute mainstream smoke, and any toxicological evaluation should respect that fact. Contrary to the U.S. EPA's assertion, a relationship between ETS exposure and lung cancer is not biologically plausible. The assumptions made by U.S. EPA (1992) do not conform to basic toxicologic principles. These assumptions fatally flawed the ability of U.S. EPA to reach an unbiased, scientifically sound conclusion as to the relationship between ETS exposure and the risk of lung cancer.

3.3. Information That Has Become Available Since Issuance of The 1992 U.S. EPA Report

Since the 1992 U.S. EPA report was written (U.S. EPA, 1992), additional relevant animal studies have been published that should be considered by Cal/EPA. New information has been developed regarding the mechanistic and toxicologic consequences of exposure to various concentrations of an ETS surrogate, aged and diluted sidestream smoke. These studies have examined toxicity and histopathology (Coggins et al., 1992, 1993; Teredesai and Pruhs, 1994), carcinogenicity (Witschi et al., 1995) and potential mechanisms of action such as cellular proliferation (Rajini et al., 1994; Ayres et al., 1995; Witschi et al., 1995), cytochrome P-450 (Gebremichael et al., 1995), DNA adduct formation (Lee et al., 1992, 1993), and chromosomal aberrations (Mohtashamipur et al., 1987; Lee et al., 1992).

Tables 2 and 3 (Appendix II) summarize the studies that have been performed in animals with an ETS surrogate, aged and diluted sidestream smoke (ADSS). The results of these studies show that at high particle concentrations of ADSS that is, ≥4 mg of particles per cubic meter (m^3), some hyperplastic changes were observed primarily in the nasal epithelium of rats. For perspective, human exposure to ETS, when it occurs, is typically less than 0.1 mg of total particulate matter/m^3; a significant portion of the particulates may not be ETS. Mice and hamsters do not exhibit these changes at the highest concentrations evaluated, 4 and 6 mg of particles/m^3, respectively. The observed hyperplastic changes in rats are fully reversible. These results suggest a reactive and adaptive response in the rats that is similar to responses in this animal model following exposure to other irritants. Studies of DNA adduct formation show increased adduct formation in ADSS-exposed rats only at a particle concentration of 10 mg/m^3. The lack of increased adduct formation at 1 mg/m^3 over a period of 90 days suggests that adduct formation at the considerably higher 10 mg/m^3 concentration is in response to overload of normal or usual metabolic pathways and/or defense mechanisms. Results of cellular proliferation studies are consistent with this interpretation. See section 6.2 for additional details.

4. Background Information in Consideration of Basic Principles

4.1 Scientific Method

The evaluation of the relationship of ETS to lung cancer should be examined in the spirit of the scientific method, which is the unbiased and controlled method of testing a hypothesis. A hypothesis is formulated, tested under well designed and properly controlled conditions, and is either confirmed or rejected on the basis of the results of observation and experimentation. The hypothesis is revised, if necessary, and verified by further observation and experimentation. Based upon all the available and relevant data, a theory is formulated that seeks to explain the process of interest.

In the case of the evaluation of ETS exposure and its relationship to lung cancer, the epidemiological evidence is not convincing and does not support a hypothesis of a causal role of ETS in lung cancer. If the hypothesis is not to be abandoned, then all remaining relevant data, which include the relevant animal studies, must be evaluated. This in turn will require scientific judgment as to which animal studies are relevant to the evaluation of ETS. The basis for this determination is consideration of the factors presented in sections 4.3 and 5.

4.2. Cause and Effect

Although an epidemiological association does not demonstrate cause and effect, many epidemiologists attempt to draw a causal inference (or judgment) based on epidemiological data. The propriety of such an approach is beyond the scope of this commentary, however, several criteria that are frequently considered in making a judgment on causation are similar for epidemiology and toxicology (Spilker, 1991). One of these criteria is the strength of the relationship or association between the exposure and the effect. For epidemiology the relative risk or odds ratio is used. The strength of the statistical relationship between ETS exposure and lung cancer in the epidemiology studies chosen by Cal/EPA is weak at best as was commented on by several presenters (i.e., W. Butler, G. Gori, M. Lavois, and M. Layard) at the Cal/EPA Workshop on March 25, 1996. A dose-response relationship is an important consideration in evaluating the strength of any relationship between an exposure and an effect. For toxicology, the strength of a relationship is represented by differences between control and test populations, trends in the data, and the statistical significance of that effect, usually at $p \leq 0.05$.

Of particular importance is the requirement that the effect be biologically plausible. The more specific the effect, the easier it should be to establish such a relationship. Difficulties arise when the observed effect is not specific to the chemical in question, but can be the

result of many independent or interdependent factors. There appears to be just such a problem with the study of ETS exposure. This is one of the many advantages of animal studies . Unlike epidemiological studies, animal studies are performed under controlled conditions, exposure can be quantified, and responses can be measured. An additional advantage of animal studies is that mechanisms of action can be investigated.

4.3. Composition of Mainstream Smoke, Sidestream Smoke, and ETS Complex Chemical Mixtures

Mainstream smoke is primarily ambient air drawn through cigarette paper and around a fire-cone that includes products of combustion (water and carbon dioxide) and products of incomplete combustion (organic constituents) (Guerin et al., 1987). The particulate matter is composed of 15-25% water and a wide variety of organic constituents. It is extremely concentrated (~1 x 10^{10} particles/cm^3 [Guerin et al., 1987]; 10.5 x 10^{12} particles/cigarette [U.S. DHHS, 1986]), and individual constituents are distributed between the particulate and vapor phases according to their solubility and volatility. See Tables 1 and 2 (Appendix II) for a comparison of selected constituents of mainstream smoke, sidestream smoke, and ETS.

Sidestream smoke differs from mainstream smoke in several ways. Sidestream smoke contains large quantities of vapor-phase water, and its higher alkalinity increases the proportion of nitrogen-containing compounds in sidestream smoke compared with mainstream smoke. The vapor-phase/particulate-phase distribution of the constituents is dependent on the degree to which sidestream smoke is diluted. Active smoking reduces the total sidestream smoke delivery, as should be expected. Fresh sidestream smoke contains 3.5 x 10^{12} particles/cigarette (U.S. DHHS, 1986) which is approximately one-third the number of particles in mainstream smoke. The particle size range of sidestream smoke is also decreased compared to mainstream smoke (see Table 1 in Appendix II).

The quantity and composition of the organic vapor phase is independent of the length of the cigarette remaining, which is in direct contrast to mainstream smoke, which becomes enriched in organic constituents with each puff. The vapor phase contains 90-95% of the nicotine in highly diluted sidestream smoke, and thus nicotine is not a good marker of particle deposition in the lungs.

ETS is comprised of aged and diluted exhaled mainstream smoke, sidestream smoke generated during the puff, and sidestream smoke generated during the smolder period between puffs. The greatest contributor to ETS is the latter. Together the sidestream smoke components contribute 85 to 90% of ETS. The particle concentration of ETS is dependent on its dilution in ambient air, and is orders of magnitude less than that of mainstream smoke (i.e., ~1.5 x 10^5 particles/cm^3 vs. ~10^{10} particles/cm^3) (Guerin et al., 1987; Rodgman, 1992). Smoking an unfiltered cigarette, a smoker inhales 15-40 mg of particles (U.S. EPA, 1992). Assuming a 50% deposition in the lung, the smoker retains ≥ 7.5 mg of mainstream smoke particles/cigarette.

In an ETS environment, a nonsmoker is usually exposed to significantly less than 0.1 mg of ETS particles/m^3 (see Table 1, Appendix II) and would inhale 0.024-0.24 mg of ETS particles in 8 hours (Scherer et al., 1990). An 11% retention of particles would result in a daily dose of ≤ 26.4 μg/day. Holcomb (1993) performed calculations based on an extensive review of indoor ETS concentrations and exposures. He estimates that maximum exposure of an adult male to ETS results in retention of 108.7 μg of ETS particles/day. A smoker by comparison retains ≥ 69 times that amount with each cigarette smoked or ≥ 1380 times that per day for 20 cigarettes smoked.

There are many factors in addition to deposition that contribute to the dose that the pulmonary tissues receive. These additional factors, only a few of which are mentioned in section 5.5, would further significantly decrease the total dose of ETS particulate matter in

the lungs of nonsmokers relative to the dose of mainstream particulate matter that smokers would receive. Gori and Mantel (1991) estimate that the actual tissue dose to a nonsmoker exposed to ETS may be less than 1/10,000 of the tissue dose that is received by a smoker.

5. ISSUES FOR CONSIDERATION

5.1. Utility of Animal Studies in Evaluation of Carcinogenicity and Determination of Mechanisms of Action

There are a variety of confounding factors in epidemiology studies, such as diet and lifestyle, in addition to the great underlying genetic variability. All of these may be associated with an observed effect independent of the chemical exposure under investigation.

Animal studies offer the opportunity to control conditions that were not controlled effectively or consistently in the epidemiological studies of ETS, and to identify what if any effects are produced at a range of concentrations that more appropriately represents the human exposure to ETS. Animal studies can provide information that is not readily available from epidemiology studies, such as measurements of cellular proliferation, chromosomal aberrations, DNA adduct formation, and evaluation of histopathology.

There has been some discussion of the utility of animals for the study of the carcinogenic effects of cigarette smoking, generally because of inconsistency in identifying in rodents significant increases in the numbers and kinds of tumors that have been associated with cigarette smoking in humans (Henry and Kouri, 1986; IARC, 1986). Pulmonary (bronchogenic) squamous cell carcinoma, the tumor type reported to be most closely associated with cigarette smoking in humans, has not been observed in either mice or hamsters exposed via inhalation to mainstream smoke, sidestream smoke, or ETS (Henry and Kouri, 1986; IARC, 1986; Huber, 1989; Rodgman, 1992). Of all the inhalation studies

performed, pulmonary squamous cell carcinoma has been observed in only one rat (F344) following exposure to cigarette smoke (Dalbey et al., 1980). The study was repeated two times (Heckman and Dalbey, 1982; Heckman and Lehman, 1985), but the initial observations were not confirmed.

In spite of the relative lack of tumorigenic response of rodents to high concentrations of cigarette smoke, rodents may in fact be appropriate models of the carcinogenic potential of cigarette smoke and ETS. Factors in favor of using certain strains of mice for the study of cigarette smoke (Henry and Kouri, 1986) and ETS or its surrogate, include these:

- Lung aryl hydroxylases are induced in response to smoke exposure
- Sister chromatid exchanges in bone marrow cells are increased following smoke exposure
- DNA repair capacity is inhibited approximately 50% in the lungs of smoke-exposed mice
- DNA synthesis following smoke exposure is increased up to twenty fold
- High incidences of squamous cell carcinoma can be produced with known chemical carcinogens

5.2. Mechanisms of Carcinogenesis

The mechanisms of carcinogenesis are not well understood. There are a number of hypotheses to explain various aspects of carcinogensis, but there is much about this complex process that is unknown. There are so many influences on the process that a carcinogen must be defined with respect to species, age, dose, route and frequency of administration, age, and other factors.

Chemical carcinogenesis is the complex, multistage process in which a chemical or its metabolite disrupts normal cell growth and regulation. An understanding of this process is

important when evaluating the carcinogenic potential of a chemical mixture such as ETS, because carcinogenesis is not a simple "one-hit" process. Exposure to a mixture that contains a "carcinogenic" chemical does not guarantee that cancer will develop.

The multistep nature of carcinogenesis has been explained by a number of researchers in terms of stages: initiation, promotion, and progression. Multiple steps may in turn be present within each stage (Trosko and Chang, 1988; IARC, 1992; Barrett, 1993). Three of the processes currently recognized to be important in chemically-induced initiation of carcinogenesis are: metabolism to a reactive chemical species, DNA repair, and cell proliferation (Ames et al., 1993). Mutations, for example, transitions, transversions, and deletions, occur as a result of endogenous metabolic processes, radiation, and exogenous chemicals. Chemical initiators interact with DNA either directly or indirectly through an active metabolite to effect the above changes. Initiation is irreversible because the mutation becomes "fixed" or permanent as a result of DNA synthesis and cell division (mitosis); that is, the genotype and/or phenotype of the initiated cell is established. At this stage in carcinogenesis, any change may be subtle; neoplasia may not result because of apoptosis (programmed cell death) or because promotion and/or progression do not follow initiation (Pitot and Dragan, 1996).

Promotion is a reversible process that inhibits apoptosis and/or enhances or represses gene expression. Gene expression is altered primarily through perturbation of signal transduction pathways (Pitot and Dragan, 1996). One hypothesis is that promoting agents act through specific receptors and that the promoter's effect is directly proportional to the number of receptors that it occupies.

Another hypothesis is that promoting agents selectively increase proliferation and may also decrease apoptosis of preneoplastic cell populations. The process of promotion is subject to modulation by many factors such as diet, hormones, and age. Although understanding of

cell cycle regulation has increased dramatically in the past five years, there is still much about the process of promotion that is unknown. A promoter cannot induce cancer in and of itself; its role in carcinogenesis is dependent on an initiated population of cells.

Progression is the third stage of carcinogenesis in which complex genetic alterations occur as a result of evolving karyotypic instability. Cell proliferation has been linked to the carcinogenic process, and many chemicals that are cytotoxic at high concentrations induce regenerative cell proliferation (IARC, 1992). Increased cell proliferation can saturate the DNA repair mechanisms that correct mutations induced by normal endogenous reactive oxygen species formed during metabolism, such as super oxide anion, and peroxides. Mutations may thus be a secondary result of cellular proliferation. In addition to cellular proliferation and secondary mutagenesis, cytotoxicants may induce inflammation or may increase the levels of circulating growth factors which may preferentially increase the growth of preneoplastic cells (Butterworth et al., 1995). The presence of cell proliferation is not sufficient of itself to produce a tumor.

During this stage of carcinogenesis, additional changes occur in the genome that alter cell growth rate and responses to hormonal influences (Trosko and Chang, 1988; Pitot and Dragan, 1996). As stated by Pitot and Dragan (1996),

> "very low doses of complete carcinogens act to initiate cells but cannot sustain the remainder of the carcinogenic process. This consideration is undoubtedly very important in carcinogenesis in humans, in whom most exposures are at extremely or relatively low levels of a carcinogenic agent."

Current understanding of carcinogenesis recognizes that the probability of a chemical producing a cancer is dependent on many factors including the cell type, the site and type of chemical action within that cell, the repair capabilities of the cell, and the rate of

mitogenesis and apoptosis. Experimental studies have established that different chemicals act at various stages in the carcinogenic process. It is inappropriate to extrapolate the effects observed from a high dose to a low dose because different mechanisms of action may be active at different dose levels. See section 5.3 for further discussion.

5.3. Dose-Response Relationships

5.3.1. Relationship of Dose to the Process of Carcinogenesis

A threshold dose is a dose above which a clear response is observed or, alternatively, a dose below which no effects of interest are observed. This concept is widely accepted for noncancer toxicity endpoints, but it has been the subject of some controversy for cancer (Zeise et al., 1987; Upton, 1988; Beck et al., 1994; Sagan, 1994; Cohen, 1995; Hrudey and Krewski, 1995; Purchase and Auton, 1995). There are many factors that contribute to the ultimate expression of toxicity or disease, and disruption of any of the intermediate steps in that process can affect the observed threshold. Threshold can be explained by many factors, including the chemical failing to reach its target site or to attain a significant concentration at the target site (molecular dose). The natural capacity of a cell to repair itself also influences threshold.

A widely quoted review of dose-response relationships published by Zeise et al. (1987) was extensively used by Purchase and Auton (1995) for their discussion of thresholds in chemical carcinogenesis. They stated:

> "In the field of chemical carcinogenesis, there is a growing body of evidence to suggest that there are mechanisms of cancer induction which display the type of mechanistic threshold observed in other types of toxicity. Nongenotoxic carcinogens act by mechanisms which do not involve the direct interaction of the chemical or its metabolites with DNA."

An example they presented is the production of thyroid cancer by chemical carcinogens that interfere with thyroxin homeostasis and result in an excess of thyroid-stimulating hormone, which results in hyperplasia and eventually cancer of the thyroid. Purchase and Auton (1995) also stated that it is difficult to identify thresholds in epidemiology studies even when the dose-response relationship is sublinear. Likewise, in animal studies even an apparent threshold at low dose cannot be proven statistically because it is equivalent to proving a negative.

According to Purchase and Auton (1995), "The selection of the method of risk assessment on the basis of the presence or absence of a threshold can only be justified by consideration of mechanistic information of the toxicity under study." The effects of carcinogenic agents at very low doses become not only indistinguishable from the background incidence, but they appear insignificant when set in the context of the risks associated with other accepted societal activities.

5.3.2. High-Dose Extrapolation

The effects that are observed following high-dose exposure to chemicals or chemical mixtures are not necessarily representative of the effects that would be seen following lower doses, because the mechanisms of action leading to the observed effects at high doses may not be in action at lower doses. This is especially true for chemicals that are cytotoxic or mitogenic at high doses. In addition, normal metabolic pathways can be overwhelmed when confronted with massive amounts of a chemical. The concept of metabolic overload and its consequences have been discussed in various ways by a number of authors (Faccini et al., 1992; Wynder and Williams, 1992; Sagan, 1994). When overload occurs, the chemical concentration in the body continues to increase because the excretion pathways are saturated and/or new pathways that do not normally handle this type of chemical become involved. When new metabolic pathways are utilized, toxic metabolites

may be formed. In addition, the chemical may interact with some other macromolecule, such as a protein, or an endogenous sulfhydryl, such as glutathione. The interaction with the protein may be directly toxic by inactivating an enzyme; the interaction with glutathione may not be toxic in an of itself, but it may deplete the cell of this protective molecule and thus prime the cell for future injury by another, or more of the same, chemical.

Thus, at high doses there may be mechanisms in action that are not at all reflective of the mechanisms of chemical action at lower doses. Consequently, observations made at high doses cannot be directly extrapolated to lower doses.

Even if ETS were dilute mainstream smoke, which it is not, mainstream smoke studies are inappropriate to determine the likely effects of ETS simply because of significant differences in dose. In the case of ETS, there are additional reasons to reject observations made with high-dose sidestream smoke and mainstream smoke studies because the mixtures are also different from ETS in the concentration and distribution of their relative components.

5.3.2. Adaptive Repair

Recent discussion about the ability of an organism to respond to an insult has focused on whether a response should be described as a "toxic" response in all cases or whether the response could better be described as an "adaptive" response (Burger et al., 1989; Calabrese, 1992; Farber, 1992; Sagan, 1994). Burger et al. (1989) have addressed this question for laryngeal squamous cell metaplasia, changes in goblet cells of the nasal epithelium, macrophage accumulation within alveoli, and bronchiolization of the alveolar epithelium. In their review they have summarized the observations and conclusions of several pathologists and have come to the conclusion that the above-mentioned responses can indeed be adaptive responses that are not preneoplastic. Several authors have noted

that exposure to low levels of radiation or chemicals can produce adaptive effects that are protective against exposure at higher levels (Calabrese, 1992; Farber, 1992; Sagan, 1994).

5.3.3. Other Sources of Mutagens and Animal Carcinogens Reported to be in Mainstream Smoke, Sidestream Smoke, or ETS

Mutagens and animal carcinogens reported to be present in mainstream smoke, sidestream smoke, or ETS are reported to have many sources, with the possible exception of what have been called the tobacco-specific nitrosamines. Humans are routinely exposed to chemicals, principally via inhalation of airborne material; ingestion of food, beverages, and water; and dermal absorption of handled materials, soils, and radiation. Several approaches have been used to assess human exposure, including the U.S. EPA's Total Assessment Exposure Assessment Methodology (TEAM), which used direct measurement for multimedia studies (Waldman et al., 1991) and the Total Human Environmental Exposure Study (THEES) (Waldman et al., 1991). These studies will not be discussed here, but benzo[a]pyrene will be used as an example of a pollutant present in everyday life.

The class of compounds known as polyaromatic hydrocarbons (PAHs) are of interest because of their reported presence in mainstream smoke and ETS. Benzo[a]pyrene was the PAH chosen for measurement in THEES (Waldman et al., 1991). This study identified that in outdoor air the ambient concentrations of benzo[a]pyrene strongly influenced total inhalation exposure. Additionally, cooking activities, combustion appliances, and cigarette smoke were named as important sources of indoor air exposures. Dietary exposure to benzo[a]pyrene in this study ranged from 2 to 500 ηg per day, which was greater than daily inhalation of 10 to 50 ηg per day. Hattemer-Frey and Travis (1991) state that,

> "the food chain is the dominant pathway of human exposure, accounting for about 97% of the total daily intake of BaP [benzo[a]pyrene]. Inhalation and consumption

> of contaminated water are only minor pathways of human exposure. The long-term average daily intake of benzo[a]pyrene by the general population of the U.S. is estimated to be 2.2 micrograms (μg) per day. Cigarette smoking and indoor activities do not substantially increase human exposure to BaP relative to exposures to background levels of BaP present in the environment."

The heating of certain foodstuffs, such as broiled meat and fish, are known to significantly increase the PAH content of the food as well as the indoor air (Waldman et al., 1991). A single 8-ounce serving of charcoal-broiled T-bone steak could provide 420 to 716 mg of benzo[a]pyrene which is ~ 9 to 143 times the amount in the mainstream smoke from one cigarette and up to over 700 times the amount that would be inhaled in ETS over an 8-hour period (IARC, 1983).

5.5. Inhalation, Deposition, and Absorption of Materials

There are many factors that influence the total dose that the lung will experience. Some of these factors are species, age, sex, respiratory rate, tidal volume, mucociliary clearance, permeability of the alveolar-capillary carrier, activity and number of pulmonary macrophages, etc. A few factors from this list are discussed below as examples of the differences between the smoker and the nonsmoker. Not only are cigarette smoking and ETS inhalation extremely different activities and mainstream smoke and ETS very different complex mixtures, the smoker and the nonsmoker are themselves physiologically quite different.

5.5.1. Retention of Mainstream Smoke and ETS Particles

In determining the potential toxicity of an agent, it is important to evaluate the total amount of chemical that is delivered to an animal and the dose that is actually retained and ultimately absorbed. Differences between ETS and mainstream smoke in their deposition

and retention provide another reason for not extrapolating the effects of one to the other. For active smokers the percentage of particles from mainstream smoke that is deposited in the lung reportedly ranges between 50 to 90% (Gori and Mantel, 1991; U.S. EPA, 1992). In contrast, approximately 11% (Hiller, 1984) to 43% (McAughey et al., 1994) of the particles from ETS are deposited in the lungs of nonsmokers. Because the number of particles in mainstream smoke is significantly higher than in ETS, the active smoker receives a much greater unit dose and daily dose of particles and their associated chemicals than the nonsmoker does. Thus, studies of mainstream smoke are not studies of ETS and the observations made in these studies cannot be extrapolated to ETS.

5.5.2 Activity of Clearance Mechanisms

There are three main ways in which materials deposited in the lung are removed: mucociliary transport up the airway tree; dissolution into ions, atoms, or molecules that are transported in the blood; and drainage to lung-associated lymph nodes (Valberg and Blanchard, 1991). Clearance from the lung interstitium is via translocation to the lung lymph nodes, which in turn are cleared very slowly. For "insoluble" materials, the mucociliary pathway is most active 0 to 48 hours after exposure. It clears particles on the mucus lining of the ciliated airways. Smaller animal species have slower velocities of clearance than larger species. This being true, one would expect that data collected from rodent exposure might overestimate human toxicity.

The alveolar macrophage is another means for the lung to clear debris. Once particles are engulfed, macrophages are then cleared via transport up the mucociliary pathway. The calculated number of alveolar macrophages per alveolus is 0.037 for mice, 0.11 for rats, and 6.8 for humans; or, put another way, the area patrolled by each alveolar macrophage is 190,000, 140,000, and 22,000 μm^2, respectively (Valberg and Blanchard, 1991). From the

numbers, one can surmise that alveolar macrophages will not play as prominent a role in the clearance of particles from the lungs of rodents as from the lungs of humans.

5.5.3. Instillation Studies in Animals

Some of the most significant effects that have been produced related to mainstream and sidestream smoke are related to the instillation or implantation of concentrated forms of the two smokes (Wynder and Hoffmann, 1967). In some studies, reported animal carcinogens that are components of mainstream smoke have been instilled at very high concentrations into the lungs of animals (IARC, 1986). Instillation of many chemicals is known to produce an inflammatory reaction (Valberg and Blanchard, 1991). Aviado (1995) comments that "the animal model has lost defensive mechanisms to prevent absorption in the bronchopulmonary system" which are normally present and protect against inhaled chemicals. More importantly from a mechanistic point of view, instillation also has been observed to increase epithelial mitotic rates and to enhance hamster respiratory carcinogenesis (Valberg and Blanchard, 1991). The increased mitotic rates may be responsible for preventing normal DNA repair and for fixing mutations.

It is not surprising in the case of either instillation or implantation that the incidence of tumors is significantly increased over the control rates. In light of the earlier discussion of the mechanisms of chemical action and the process of carcinogenesis in section 5.2, it is obvious that the results of these studies are, first, inappropriate to evaluate the carcinogenic potential of mainstream and sidestream smoke, and, second, irrelevant for the evaluation of the carcinogenic potential of ETS.

5.6. Relationship of Particle Size and Toxicity

Cells in the respiratory tract are larger than mainstream smoke, sidestream smoke, or ETS particles that might settle in the respiratory tract. Adjacent cells may receive very different

doses depending on the deposition of particles on individual cells. The size of the particle that is deposited may have a significant effect on toxicity because the mass of a spherical particle is proportional to the cube of the geometric diameter. One thousand (1000) particles of 0.1 μm diameter must be deposited in the lung to equal the mass burden from the deposition of a single 1-μm diameter particle (Phalen, 1984a). The size of the particle may affect its clearance from the lung by mucociliary mechanisms or macrophages or its dissolution in the surrounding fluid. The effect on toxicity may be significant.

5.7. Knowledge of Individual Components Has No Predictive Value for Evaluating a Complex Chemical Mixtures

The effects of a complex chemical mixtures cannot be predicted from knowledge of the effects of its individual components. Interactive effects can be derived, for example, from biologic interactions within the animal or human and from physical and chemical interactions in the air, including adsorption of gases on particle surfaces, hygroscopicity, changes in particle size, etc. (Phalen, 1984b). Other factors may influence the total dose and the sites of deposition and absorbance. Irritancy and acidity are examples of factors that may influence the rate of breathing and other processes. Interactions of the individual components of a complex mixture at a cellular level may produce biologic effects that are synergistic, additive, or antagonistic. Thus, knowledge of the effects of individual components is not predictive of the effects of the mixture. The only way to evaluate the potential effects of the complex mixture known as ETS is to test ETS.

The mouse and rat have relatively higher minute volumes per gram of lung than do humans (See Table 4 in Appendix II). These rodents can potentially receive much higher doses of an inhaled chemical mixture per unit of lung weight than humans. This is important when one considers the predictability of toxicity from one species to another. In the case of ETS, proportionately the mouse and the rat could potentially receive approximately 16 times the

inhaled dose that a human male would receive (0.105/0.006 = ~16). Of course, many other factors are operative, such as the differences in the nasal pharyngeal anatomy between rodents and humans. The primary disadvantage in inhalation studies with rats and mice, in fact, derives from their short, relatively wide airways and their tendency to lack respiratory bronchioles (Phalen, 1984c). In spite of this shortcoming, the fact that mice and rats are obligate nose-breathers, and the fact that their nasal turbinates are significantly different from humans', there is no reason to suspect that either a mouse or a rat would receive less of a dose to the lung than would a human.

In addition to the animal's breathing pattern and the properties of an inhaled material, the anatomy of the respiratory system airspaces will determine how much of a pollutant will initially deposit within the subject and where it will be deposited. Typically, the mechanisms of diffusion, sedimentation, and impaction are important in particle deposition. (Phalen, 1984c)

6. Evaluation of Animal Studies Relevant to ETS

6.1. Aged and Diluted Sidestream Smoke (ADSS) as a Surrogate for ETS

Although it is preferable to study ETS directly, there are practical limitations that arise from its dilute nature. For this reason, aged and diluted sidestream smoke was developed as a surrogate for ETS. ADSS only lacks the exhaled mainstream smoke component and, thus, is the best surrogate for ETS. Aviado (1995) states, "This author, after review of animal studies, has concluded that an inhalation technique using aged and diluted SSS [sidestream smoke] is the only acceptable procedure by which to obtain data that are relevant to a claim that ETS is a pulmonary carcinogen." Cal/EPA should give special attention to those animal studies that have been performed utilizing aged, diluted

sidestream smoke as a surrogate for ETS because they provide the best available means for the evaluation of ETS toxicity at concentrations that are relevant for human exposure.

6.2. Summary of Observations Made in Studies with ADSS

Of publications in which ADSS was used, 11 were identified as being relevant for the evaluation of ETS pulmonary toxicity and carcinogenicity. Tables 4 and 5 (Appendix II) summarize the concentrations, evaluated endpoints, and results of these studies. Additional information may be found in the abstract for each article (included in section 6.3). The original articles for these studies are included in Appendix III.

For ease of evaluation, the studies have been divided into two tables, one of toxicology studies and the other of mechanistic studies. A single publication may be represented in both tables.

6.2.1 Inhalation Studies of Toxicity and Carcinogenicity

In two studies, inhalation of high concentrations of ADSS (i.e., 4 and 10 mg/m^3) has been shown only to produce reversible, slight to mild epithelial hyperplasia and inflammation of the nasal cavity. Von Meyerinck et al. (1989) exposed rats and hamsters to 4 mg/m^3 of ADSS for 10 hours a day, 5 days a week for 90 days. Coggins et al. (1993) exposed rats to 0.1, 1.0, or 10 mg/m^3 of ADSS for 6 hours a day, 5 days a week for 90 days.

The recovery phase that was designed in each study provides significant information relevant to the mechanism of toxicity and the potential for carcinogenicity. The reversibility of effects at the extremely high particle concentrations of 4 and 10 mg/m^3 strongly suggests that the observed changes were reactive and adaptive responses to repeated irritation rather than toxic responses *per se* (Burger et al., 1989).

No histopathological changes were observed by von Meyerinck et al. (1989) in ADSS exposed hamsters. Especially in light of a lack of effects in hamsters and a reversible effect in rats, it is indeed unfortunate that U.S. EPA omitted the work of von Meyerinck et al. (1989) from its 1992 report.

The findings of these investigators (von Meyerinck et al., 1989; Coggins et al., 1993) are further supported by a recently published study by Witschi et al. (1995). A/J mice were exposed by inhalation for a 6-month period to 4 mg/m^3 of ADSS for 6 hours per day, 5 days per week. Researchers in that study observed no effects of ETS exposure on tumor incidence. These observations are significant and interesting because the A/J mouse strain is known to be a sensitive test system that exhibits tumors within 6 months in response to a number of carcinogens.

6.2.2 Inhalation Studies of Cellular Proliferation

The results of cellular proliferation studies performed in mice by Rajini et al. (1994) and Witschi et al. (1994, 1995) and in rats by Ayres et al. (1995) are consistent with the above histopathological observations. Results of the studies of cellular proliferation are consistent with the presence of reactive and adaptive tissue responses. Similar to the observed histopathological changes, the proliferative changes are reversible.

6.2.3. Inhalation Studies of DNA Adduct Formation

Studies of DNA adduct formation show increased adduct formation in ADSS-exposed rats (Lee et al., 1992, 1993) only at a particle concentration of 10 mg/m^3. The lack of increased adduct formation at 1 mg/m^3 over a period of 90 days suggests that adduct formation at the considerably higher 10 mg/m^3 concentration is in response to overload of normal or usual metabolic pathways and/or defense mechanisms. It is well recognized that a DNA adduct is not equivalent to a tumor (Swenberg et al., 1990). Adduction only informs the

investigator that something has happened within a cell. Without sophisticated techniques it is difficult to identify the adduct and to know its exact source. Adduct formation *per se* is not predictive of outcome. In the case of ETS, delivery of ADSS at concentrations over 100 fold that of a "typical" human exposure is not relevant.

6.2.4. Inhalation Studies of Cytochrome P450 and Clastogenesis

The measurement of lung cytochrome P450s 1A1 and 2B1 activities following exposure of rats to ADSS (Gebremichael et al., 1995) demonstrate that P4501A1 can be induced. The significance of this observation for humans exposed to ETS is unclear because of the high exposure to ADSS (i.e., 1.0 mg/m3).

The observation of clastogenesis by Mohtashamipur et al. (1987) has not been confirmed. The methodology used is inadequately documented. The exposure conditions and concentrations are unclear and the experimental design and methodology appear to be inappropriate. There is insufficient information to evaluate this paper.

6.2.5. Summary of Inhalation Studies with ADSS

A review of the most relevant studies in which animals were exposed via inhalation to ADSS, the most appropriate surrogate for ETS, reveals no association between ETS exposure and lung cancer. Any observed changes are consistent with reactive and adaptive responses. Abstracts of each of the studies follow in section 6.3.

6.3. Abstracts of Studies with ADSS

6.3.1. Clastogenic effect of passive smoking on bone marrow polychromatic erythrocytes of NMRI mice (Mohtashamipur et al., *Toxicol Lett*, 1987)

> The genotoxic effect of passive inhalation of sidestream cigarette smoke on bone marrow polychromatic erythrocytes was studied using male NMRI mice.

The animals were placed in individual 145.2-dm3 glass chambers resembling a room provided with normal air flow. They were exposed to the sidestream smoke of a commercial brand of cigarettes smoked by a smoking machine under standard conditions. Increased formation of micronuclei within polychromatic erythrocytes (PCEs) of femoral bone marrow 30 h after passive smoking was regarded as being due to the clastogenic effect of the smoke. Passive inhalation of the diluted sidestream smoke of a single cigarette resulted in a significant increase (P less than 0.01) in the frequency of micronucleated PCEs. This clastogenic activity was found to be dose-dependent.

6.3.2. Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study (von Meyerinck et al., *Exp Pathol,* 1989)

A 90-day feasibility study was performed in which rats and hamsters were exposed to the sidestream smoke of cigarettes. The only histopathological changes observed were hyperplasia and metaplasia of the epithelium covering the dorsal nasal turbinate bones in rats. These effects were reversible within 90 days. [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.3. Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. I. Inhalation toxicology and histopathology (Coggins, et al., *Fundam Appl Toxicol,* 1992)

Sprague-Dawley rats were exposed 6 hr per day for 14 consecutive days to aged and diluted sidestream smoke (ADSS), used as a surrogate for Environmental Tobacco Smoke (ETS), at concentrations of 0.1 (typical), 1 (extreme), or 10 (exaggerated) mg of particulates per cubic meter. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End-points included histopathology, CO-oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The only pathological response observed was slight to mild epithelial hyperplasia and inflammation in the most rostral part of the nasal cavity, in the high-exposure group only. No effects were noted at medium or low exposures. The minimal changes noted were reversible, using a subgroup of animals kept without further treatment for an additional 14 days. Overall, the end-points used in the study demonstrated that there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations and that the activity was only minimal at very exaggerated concentrations (particle concentrations 100 times higher than typical real-world concentrations). [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.4. Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. II. DNA adducts and alveolar macrophage cytogenetics (Lee et al., *Fundam Appl Toxicol,* 1992)

> The chemical constituents of cigarette smoke are greatly diluted in environmental tobacco smoke (ETS). In the typical indoor environment where cigarettes are smoked, the mean value of respirable suspended particles is approximately 0.1 mg/m3. In this study, we used aged and diluted sidestream smoke (ADSS) of 1R4F University of Kentucky research cigarettes as a surrogate for ETS and exposed Sprague-Dawley rats nose-only to 0, 0.1, 1.0, and 10 mg wet total particulate matter (WTPM)/m3 for 6 hr per day for 14 consecutive days. DNA from lung, heart, larynx, and liver was tested for adduct formation after 7 and 14 days of exposure and after 14 days of recovery. In addition, alveolar macrophages from animals exposed for 7 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any of the animals at 0.1 or 1.0 mg WTPM/m3, which represent ambient and 10-fold exaggerated ETS concentrations, respectively. Slight diagonal radioactive zones, characteristic of adducts observed in human smokers and in animals exposed to mainstream smoke, were observed, but only in lung and heart DNA of animals exposed to the highest concentration of ADSS (10 mg WTPM/m3), a 100-fold exaggeration of typical field measurements of ETS. The mean relative adduct labeling values (+/- SE) were 8.7 (+/- 0.2) adducts per 10(9) nucleotides for lung DNA and 5.7 (+/- 0.7) adducts per 10(9) nucleotides for heart DNA after 14 days of exposure. No elevation in chromosomal aberrations was observed in alveolar macrophages.(ABSTRACT TRUNCATED AT 250 WORDS) [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.5. Subchronic inhalation study in rats using aged and diluted sidestream smoke from a reference cigarette (Coggins et al., *Inhal Toxicol,* 1993)

> Male Sprague-Dawley rats were exposed 6 hr/day, 5 days/week for up to 13 weeks to aged and diluted sidestream smoke (ADSS), used as a surrogate for environmental tobacco smoke (ETS), at concentrations of 0.1 ("typical"), 1 ("extreme"), or 10 ("exaggerated") mg of particulates/m^3. Subgroups of animals were killed after 1 and 4 weeks of exposure. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End points included histopathology, CO oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The target particulate concentrations were achieved; at the exaggerated exposure they resulted in CO concentrations in excess of 50 ppm. Particle size distributions showed that the aerosols were completely respirable: the mass median

diameter values were less than 1 μm. The only pathological response observed was slight to mild epithelial hyperplasia in the rostral nasal cavity, in the exaggerated exposure group only. No effects were noted at low (typical of measured real-world ETS concentrations) or extreme exposures. The changes were similar in animals killed after 4, 28, or 90 days, and were also similar to those noted in an earlier experiment with only 14 days duration, indicating that the change does not progress with increased exposure duration from 4 to 90 days. The nasal change was absent in a subgroup of animals kept without further smoke exposure for an additional 90 days, indicating complete reversibility. Overall, the end points used in the study demonstrated that (1) there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations, and (2) the activity was only minimal at exaggerated concentrations in one region of one organ only. Based on the nasal histopathology, the NOEL for the 90-day study is >1 mg/m^3.

6.3.6. Ninety-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette: DNA adducts and alveolar macrophage cytogenetics (Lee et al., *Fundam Appl Toxicol.* 1993)

To study the genotoxic effects of subchronic exposure to environmental tobacco smoke, Sprague-Dawley rats were exposed to 0, 0.1, 1.0, and 10 mg total particulate matter (TPM)/m3 of aged and diluted sidestream smoke (ADSS) from 1R4F reference cigarettes 6 hr per day, 5 days a week for 13 weeks. DNA from lung, heart, larynx, bladder, and liver was tested for adduct formation by the 32P-postlabeling assay after 28 (except bladder) and 90 days of exposure and 90 days after cessation of exposure. In addition, alveolar macrophages from animals exposed for 28 or 90 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any tissue in any of the animals exposed to 0.1 or 1.0 mg TPM/m3. However, increased levels of DNA adducts with diagonal radioactive zones were observed in lung, heart, and larynx DNA of animals exposed to the highest concentration of ADSS (10 mg TPM/m3). Adduct analyses with varying amounts of DNA from lungs of mid- and high-exposure animals clearly indicate that the dose-response for DNA adduct To study the genotoxic effects of subchronic exposure to environmental formation is nonlinear. The adduct levels were highest after 90 days of exposure and were significantly reduced in all target tissues 90 days after cessation of exposure. Chromosomal aberrations in alveolar macrophages were not elevated in any group after 28 or 90 days of exposure. These results indicate a no-observed-effect-level (NOEL) of at least 1.0 mg/m3 for DNA adduct formation in lung, heart, and larynx, and a NOEL of at least 10 mg/m3 for the induction of chromosome aberrations in alveolar macrophages, under the conditions of this study. [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.7 Histopathological findings in the rat and hamster respiratory tract in a 90-day inhalation study using fresh sidestream smoke of the standard reference cigarette 2R1 (Teredesai and Pruhs, *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract,* ICSI Press, Washington, DC, 1994)

The reserve cell hyperplasia of the rat nasal respiratory epithelium and the lack of findings for the hamster are in accordance with published literature (von Meyerinck et al. 1989, Coggins et al. 1992, 1993). The slight hyperplasia and the slight squamous metaplasia found in the rat laryngeal epithelium have not been reported to date in the literature. The changes were reversible and are considered to be an adaptive response to repeated irritation. The No Observed Effect Level (NOEL) for all FSS-related findings for this study is between 2 and 6 μg TPM/L for rats. This concentration range is between 1 and 2 orders of magnitude above the average environmental concentration.

6.4.8. Short-term effects of sidestream smoke on respiratory epithelium in mice: cell kinetics (Rajini and Witschi, *Fundam Appl Toxicol,* 1994)

Male strain A/J and C57BL/6 mice were exposed on five consecutive days, for 6 hr a day, to sidestream smoke generated from Kentucky 1R4F reference cigarettes. Chamber concentrations were 1 mg/m3 of total suspended particulate matter and 528 to 549 micrograms/m3 of nicotine. Cumulative labeling indices in the airways and in the pulmonary parenchyma were measured following 1, 3, or 5 days exposure to Earlier studies have shown that both mainstream and sidestream cigarette smoke increase the activities of cytochrome P4501A1 and 2E1 in the lungs of adult animals; however, little information is available on the influence of ambient levels of sidestream cigarette smoke on cytochrome P450 monooxygenase activity in the developing lung. The present studies were conducted to define the developmental profiles of cytochrome P450 monooxygenases 1A1 and 2B1 in rat lung and liver and to assess the effects of aged and diluted sidestream cigarette smoke (ADSS) on the developmental profile of these two enzymes. Accordingly, pulmonary and hepatic microsomal P4501A1 and 2B1 activities were determined by measuring ethoxy- and pentoxyresorufin-O-delakylase (EROD and PROD, respectively) activity in animals exposed to filtered air or ADSS from birth to 7, 14, 21, 50, and 100 days of age. Pulmonary P4501A1 activity in control rats was not detected until 14 days of age. Activities increased threefold between 14 and 21 days of age and remained unchanged to 100 days of age. In animals exposed to ADSS from birth, pulmonary EROD activities were detected as early as 7 days postnatal and were elevated

three- to fourfold above control at all other ages examined. Hepatic EROD activities were unaltered by ADSS exposure. Short-term (4-day) ADSS exposure was as effective in upregulating pulmonary microsomal EROD activities as 100 unfiltered or filtered sidestream smoke. A significantly increased labeling index was found in A/J mice in the epithelium lining large intrapulmonary airways and terminal bronchioles after 3 and 5 days exposure to unfiltered smoke, whereas following exposure to filtered smoke labeling indices remained normal. The alveolar labeling index was not increased following smoke exposure. In C57BL/6 mice, sidestream smoke did not produce signs of increased cell proliferation in the respiratory tract. It is concluded that the response to sidestream smoke inhalation in mice may depend upon the strain of mice

6.3.9. Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data (Witschi, et al., *Fundam Appl Toxicol,* 1995)

Male strain A/J mice were exposed to sidestream smoke (SS) generated from burning Kentucky 1R4F reference cigarettes. Chamber concentrations were 4 mg/m3 of total suspended respirable particulate matter (TSP). Animals were exposed 6 hr a day, 5 days a week. One-week cumulative labeling indices were significantly increased in the large intrapulmonary airways during the 1st week and in the respiratory epithelium of the nasal and maxillar turbinates during the first 3 weeks of exposure and then returned to control values. Subsequently, signs of increased cell proliferation were again found in the nasal and maxillar turbinates during the 9th and 16th exposure weeks. The experiment was terminated after 6 months. The number of animals bearing lung tumors was the same in smoke-exposed as in filtered air-exposed animals as was the average number of tumors per lung. Analysis of the DNA of individual tumors obtained from exposed and control mice for K-ras mutations suggested that exon 2 might be a specific target for SS. It was concluded that (1) duration of exposure was too short or (2) concentration of TSP was too low to reveal a possible carcinogenic potential of SS in strain A/J mice or that (3) SS is not carcinogenic in strain A mice.

6.3.9. Replicative DNA synthesis in tissues of the rat exposed to aged and diluted sidestream smoke (Ayres et al., *Inhalation Toxicology,* 1995)

Male Sprague-Dawley rats were exposed to aged and diluted sidestream smoke (ADSS) from Kentucky 1R4F reference cigarettes for 6 h/day, 5 days/wk, for a 13-wk period. Exposure concentrations were 0, 0.1, 1, and 10 mg ADSS/m^3. Exposures were conducted in whole-body inhalation chambers. Rats were held in nose-only exposure tubes for the 6-h exposures to minimize pelt deposition and subsequent ingestion of ADSS. Groups of 10

rats from each exposure group were killed after 5, 28, and 90 d of exposure to examine the rates of replicative DNA synthesis; 6 rats from each exposure group were kept for a 90-day recovery period after termination of exposures to examine replicative DNA synthesis rates. Three days prior to each scheduled necropsy, an osmotic pump containing 5-bromo-2'-deoxyuridine (BrdU) was implanted subcutaneously. After necropsy, tissues were processed for examination of BrdU-containing cells at several sites. Incorporation of BrdU was assessed either by counting the number of labeled cells along a length of an epithelial surface or by counting the number of labeled cells in an area of tissue. Tissues examined were from the nasal cavity, ventral larynx, and trachea, in addition to bronchial, bronchiolar, and alveolar regions of the lung. Endocardium, myocardium, epicardium, and aortic smooth muscle sites were also examined. Increased replicative DNA synthesis occurred in some sites of the respiratory tract at the 5-day time point at the mid or high exposure concentrations, although at 28 and 90 days, these effects had diminished in intensity or were not present, indicating adaptation to the ADSS exposure. The only tissues with elevated rates of replicative DNA synthesis at 90 days were the cuboidal and respiratory epithelium at the most rostral portion of the nasal cavity at the highest exposure concentration. Increased rates of replicative DNA synthesis were not noted in heart tissues or lung alveolar epithelium at any concentration at any time point. Examination of rats killed after the end of the 90-day recover period indicated that the increase in replicative DNA synthesis was not sustained after termination of exposures. The no observed effect level (NOEL) for increased replicative DNA synthesis after subchronic exposure to ADSS in the rate is greater than 1 mg ADSS/m^3

6.3.11. Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: effect of aged and diluted sidestream cigarette smoke (Gebremichael, et al., *Toxicol Appl Pharmacol,* 1995)

Earlier studies have shown that both mainstream and sidestream cigarette smoke increase the activities of cytochrome P4501A1 and 2E1 in the lungs of adult animals; however, little information is available on the influence of ambient levels of sidestream cigarette smoke on cytochrome P450 monooxygenase activity in the developing lung. The present studies were conducted to define the developmental profiles of cytochrome P450 monooxygenases 1A1 and 2B1 in rat lung and liver and to assess the effects of aged and diluted sidestream cigarette smoke (ADSS) on the developmental profile of these two enzymes. Accordingly, pulmonary and hepatic microsomal P4501A1 and 2B1 activities were determined by measuring ethoxy- and pentoxyresorufin-O-delakylase (EROD and PROD, respectively) activity in animals exposed to filtered air or ADSS from birth to 7, 14, 21, 50,

and 100 days of age. Pulmonary P4501A1 activity in control rats was not detected until 14 days of age. Activities increased threefold between 14 and 21 days of age under manned unchanged to 100 days of age. In animals exposed to ADSS from birth, pulmonary EROD activities were detected as early as 7 days postnatal and were elevated three- to fourfold above control at all other ages examined. Hepatic EROD activities were unaltered by ADSS exposure. Short-term (4-day) ADSS exposure was as effective in upregulating pulmonary microsomal EROD activities as 100-day exposures. Induction of pulmonary EROD activities and the associated increases in mRNA levels were dependent upon the particulate fraction. Stimulation of EROD activities in major and minor daughter subcompartments was there- to fourfold higher in ADSS-exposed animals compared to controls, while there was no induction in the trachea and less than a twofold increase in the parenchyma. Pulmonary PROD activities developed more slowly than EROD and did not reach adult levels until Day 50. ADSS did not alter pulmonary or hepatic PROD activities. These studies show that P4501A1 and 2B1 develop at different rates in rat lung and liver and that exposure to ADSS markedly increases P4501A1 activities in the lung at all ages examined.

7. REFERENCES

Ames BN, Shigenaga MK, Gold LS (1993). DNA lesions, inducible DNA repair, and cell division: Three key factors in mutagenesis and carcinogenesis. *Environmental Health Perspectives* **101**: 35-44

Aviado DM (1995). Occupational lung disease and environmental tobacco smoke. *Internaional Journal of Occupational Medicine and Toxicology* **4**: 383-407

Ayres PH, McKarns SC, Coggins CRE, Doolittle DJ, Sagartz JE, Payne VM, Mosberg AT (1995). Replicative DNA synthesis in tissues of the rat exposed to aged and diluted sidestream smoke. *Inhalation Toxicology* 7: 1225-1246

Barrett JC (1993). Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Environmental Health Perspectives* **100**: 9-20

Beck BD, Rudel R, Calabrese EJ (1994). The use of toxicology in the regulatory process. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, Ltd, pp 28-40

Burger GT, Renne RA, Sagartz JW, Ayres PH, Coggins CRE, Mosberg AT, Hayes AW (1989). Histologic changes in the respiratory tract induced by inhalation of xenobiotics: physiologic adaptation or toxicity? *Toxicology and Applied Pharmachology* **101**: 521-542

Butterworth BE, Conolly RB, Morgan KT (1995). A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancer Letters* **93**: 129-146

Calabrese EJ, ed. (1992). Bilogical effects of low level exposures to chemicals and radiation. Boca Raton: Lewis Publishers

Coggins CRE, Ayres PH, Mosberg AT, Ogden MW Sagartz JW, Hayes AW (1992). Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. I. Inhalation toxicology and histopathology. *Fundamental and Applied Toxicology* **19**: 133-140

Coggins CRE, Ayers PH, Mosberg AT, Sagartz JW, Hayes AW (1993). Subchronic inhalation study in rats using aged and diluted sidestream smoke from a reference cigarette. *Inhalation Toxicology* **5**: 77-96

Cohen BL (1995). How dangerous is low level radiation? *Risk Analysis* **15**: 645-653

Dalbey WE, Nettesheim P,Griesemer R, Caton JE, Guerin MR (1980). Chronic inhalation of cigarette smoke by F344 rats. *Journal National Cancer Institute* **64**: 383-390

Faccini JM, Butler WR, Friedmann JC, Hess R, Reznik, Ito N, Hayashi Y, Williams GM (1992). IFSTP guidelines for the design and interpretation of the chronic rodent carcinogenicity bioassay. *Experimental Toxicology and Pathology* **44**: 443-456

Farber E (1992). Cellular adaptation as an important response during chemical carcinogenesis. In: Calareses EJ, ed. Bilogical effects of low level exposures to chemicals and radiation. Boca Raton: Lewis Publishers, pp 113-118

Gebremichael A, Chang AM, Buckpitt AR, Plopper CG, Pinkerton KE (1995). Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: effect of aged and diluted sidestream cigarette smoke. *Toxicology and Applied Pharmacology* **135**: 246-253

Gori GB, Mantel N (1991). Mainstream and environmental tobacco smoke. *Regulatory Toxicology and Pharmacology* **14**: 88-105

Guerin MR, Higgins CE, Griest WH (1987). The analysis of the particulate and vapour phases of tobacco smoke. In: O'Neill IK, Burnnemann KD, Dodet B, Hoffman D, eds. Environmental carcinogens methods of analysis and exposure measurement. Volume 9 Lyon: International Agency for Research on Cancer, pp 115-139

Guerin MR, Jenkins RA, Tomkins BA (1992). The chemistry of environmental tobacco smoke: composition and measurement. Boca Raton: Lewis Publishers

Guerin MR (1987). Formation and physicochemical nature of sidestream smoke. In: O'Neill IK, Brunnemann KD, Dodet B, Hoffmann D, eds. Environmental carcinogens methods of analysis and exposure measurement. Lyon: International Agency for Research on Cancer, pp 11-23

Hattermer-Frey HA, Travis CC (1991). Benzo-a-pyrene: environmental partitioning and human exposure. *Toxoicology and Industrial Health* 7: 141-157

Heckman CA, Dalbey E (1982). Pathogenesis of lesions induced in reat lung by chronic tobacco smoke inhalation. *Journal of National Cancer Organization* **1**: 117-129

Heckman CA, Lehman GL (1985). Ultrastructure and distribution of intracellular spicules in rat lung following chronic tobacco smoke exposure. *Journal National Cancer Institute* **74**: 647-657

Henry CJ, Kouri RE (1986). Chronic inhalation studies in mice. II. Effects of long-term exposure to 2R1 cigarette smoke on (C57BL/Cum X C3H/AnfCum)F1 mice. *Journal National Cancer Institute* 77: 203-212

Hiller FC (1984). Deposition of sidestream cigarette smoke in the human respiratory tract. *Preventive Medicine* **13**: 602-607

Holcomb LC (1993). Indoor air quality and environmental tobacco smoke: concentration and exposure. *Environmental International* **19**: 9-40

Hoffman D, Hecht SS (1990). Advances in tobacco carcinogenesis. In: Cooper CS, Grover PL, eds. Chemical carcinogenesis and mutagenesis I. New York: Springer-Verlag, pp 85-102

Hrudey SE, Krewski D (1995). Is there a safe level of exposure to a carcinogen? *Enviornmental Science & Technology* **29**: 370-375

Huber GL (1989). Physical, chemical, and biologic properties of tobacco, cigarette smoke, and other tobacco products. *Seminars in Respiratory Medicine* **10**: 297-332

International Agency for Research on Cancer (IARC, 1983). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Volume 32. Lyon, France: World Health Organization

International Agency for Research on Cancer (IARC, 1986). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans: tobacco smoking, Volume 38. Lyon, France: World Health Organization.

International Agency for Research on Cancer (IARC, 1992). Mechanisms of carcinogenesis in risk identification. IARC Scientific Publications No. 116. Lyon: World Health Organization

Lee CK, Brown BG, Reed BA, Rahn CA, Coggins CRE, Doolittle DJ, Hayes AW (1992). Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. *Fundamental and Applied Toxicology* **19**: 141-146

Lee CK, Brown BG, Reed EA, Coggins CRE, Doolittle DJ, Hayes AW (1993). Ninety-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette: DNA adducts and alveolar macrophage cytogenetics. *Fundamental and Applied Toxicology* **20**: 393-401

Lee PN, Forey BA (1995). Misclassification of smoking habits as determined by continine or by repeated self-report - A summary of evidence from 42 studies. *Journal of Smoking-Related Diseases* **6**: 109-129

Lofroth G, Burton RM, Forehand L, Hammond SK, Sella RL, Zweidinger RB, Lewtas (1989). Characterization of Environmental Tobacco Smoke. *Environ Sci Technol* **23**: 610-614

McAughey JJ, Knight DA, Black A, Dickens CJ (1994). Environmental tobacco smoke retention in humans from measurements of exhaled smoke composition. *Inhalation Toxicology* **6**: 615-631

von Meyerinck L, Scherer G, Adlkofer F, Wenzel-Hartung R, Brune H, Thomas C (1989). Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study. *Experimental Pathology* **37**: 1-4

Mohtashamipur E, Norpoth K, Straeter H (1987). Clastogenic effect of passive smoking on bone marrow polychromatic erythocytes of NMRI mice. *Toxicology Letters* **35**: 153-156

National Research Council (NRC, 1986). Environmental tobacco smoke: measuring exposures and assessing health effects. Washington, DC: National Academy Press.

Phalen RF (1984a). Aerosols and gases. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 1-31

Phalen RF (1984b). Testing for toxicity. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 151-172

Phalen RF (1984c). Animal models. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 211-241

Pitot III HC, Dragan YP (1996). Chemical Carcinogenesis. In: Klaassen CD, Amdur MA, Doull J, eds. Casarett and Doull's toxicology: the basic science of poisons. New York: McGraw-Hill, pp 201-267

Purchase IFH, Auton TR (1995). Thresholds in chemical carcinogenesis. *Regulatory Toxicology and Pharmachology* **22**: 199-205

R.J. Reynolds Tobacco Company (RJR, 1986). Chemical and bilogical studies on new cigarette prototypes that heat instead of burn tobacco. Winston Salem: R. J. Reynolds Tobacco Company, pp 151-170

Rajini P, Witschi H (1994). Short-term effects of sidestream smoke on respiratory ephithelum in mice: cell kinetics. *Fundamental and Applied Toxicology* **22**: 405-410

Rodgman A (1992). Environmental tobacco smoke. *Regulatory Toxicology and Pharmacology* **16**: 223-244

Roe FJC, Perry R (1991). The physical and chemical characteristics of environmental tobacco smoke with special reference to exposure dose. In: Armitage AK, ed. Other people's tobacco smoke. E. Yorks: Galen Press, pp 21-39

Sagan L (1994). The triumph of theology over science: the non-threshold effects model. *International Journal of Occupational Medicine and Toxicology* **3**: 161-172

Scherer G, Conze C, v Meyerinck L, Sorsa M, Adlkofer F (1990). Importance of exposure to gaseous and particulate phase components of toacco smoke in active and passive smokers. *International Archives of Occupational and Environmental Health* **62**: 459-466

Spilker B (1991). Concept of cause and effect. In: Guide to clinical trials. New York: Raven Press, Ltd., pp 528-535

Sugita M, Izuno T, Kanamori M (1995). Recalculation of summarised odds ratios for the relationship between passive smoking and lung cancer based on data in the EPA report. *Indoor Environ* **4**: 177-181

Swenberg JA, Fedtke N, Fennell TR, Walker VE (1990). Relationships between carcinogen exposure, DNA adducts and carcinogenesis. In: Clayson DB, Munro IC, Shubik P, Swenerg JA, eds. Progress in predictive toxicology. New York: Elsevier Science Publishers B.V., pp 161-184

Teredesai A, Pruhs D (1994). Histopathological findings in the rat and hamster respiratory tract in a 90-day inhalation study using fresh sidestream smoke of the standard reference cigarette 2R1 In: Dungworth DL, Mauderly JL, Oberdorser G, eds. Toxic and carcinogenic effects of solid particles in the respiratory tract. Washington, D.C.: International Life Sciences Institute, pp 629-635

Trosko JE, Chang CC (1988). Nongenotoxic mechanisms in carcinogenesis: role of inhibited intercellular communication. Bradbury Report 31 Carcinogen risk assessment: new directions in the qualitative and quantitative aspects. New York: Cold Spring Harbor Laboratory, pp 139-170

U.S. Department of Health and Human Services (U.S. DHHS, 1986). Report of the Surgeon General: The Health Consequences of Involuntary Smoking. DHHS Pub No (PHS) 87-8398. Washington, D.C.

U.S. Environmental Protection Agency (U.S. EPA, 1992). Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders. Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

Upton AC (1988). Are there thresholds for carcinogenesis? The thorny problem of low-level exposure. *Annals of the New York Academy of Sciences* **534**: 563-884

Valberg PA, Blanchard JD (1991). Pulmonary macrophage physiology: origin, motility, endocytosis. In: Parent RA, ed. Comprehensive treatise on pulmonary toxicology. Boca Raton: CRC Press, pp 681-724

Waldman JM, Lioy PJ, Greenberg A, Butler JP (1991). Analysis of human exposure to benzo(a)pyrene via inhalation and food ingestion in the total human environmental exposure study (THEES). *Journal of Exposure Analysis and Environmental Epidemiology* **1**: 193-225

Witschi H, Oreffo VI, Pinkerton KE (1995). Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data. *Fundamental and Applied Toxicology* **26**: 32-40

Witshi H, Pajini P (1994). Cell inetics in the respiratory tract of hamsters exposed to sidestream smoke. *Inhalation Toxicology* **6**: 321-333

Wydner EL, Hoffman D (1967). Toabcco and tobacco smoke. New York: Academic Press

Wynder EL, Williams GM (1992). Metabolic overload and carcinogenesis from the viewpoint of epidemiology. *bga-Schriften* **3**: 17-24

Zeise L, Wilson R, Crouch EAC (1987). Dose-response relationships for carcinogens: a review. *Environmental Health Perspectives* **73**: 259-308

APPENDIX I

CURRICULUM VITAE OF SUSAN A. RICE, PH.D.

SUSAN A. RICE AND ASSOCIATES, INC.

Consultants in Toxicology and Pharmacology

CURRICULUM VITAE

SUSAN A. RICE, PH.D., D.A.B.T.

EDUCATION AND PROFESSIONAL CERTIFICATION

Diplomate of the American Board of Toxicology (1990-Present)
Ph.D. in Comparative Pharmacology and Toxicology, University of California, Davis (1976)
B.S. in Biochemistry, University of California, Davis (1971)

SUMMARY

Professional competence encompasses general toxicology and pharmacology including clinical, environmental, occupational, and product toxicology.

Areas of practice include product safety evaluation, exposure and health assessments for various routes of exposure, litigation support, and scientific support and audit for regulatory submission. Specialty areas include anesthetic toxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, reproductive toxicity, developmental toxicity, teratology (morphologic and behavioral), biotransformation of drugs and chemicals, and molecular mechanisms of toxicologic and pharmacologic action.

Agents and devices evaluated include chemicals, air pollutants (indoor and outdoor), pharmaceuticals, biologics, biotechnology products, pesticides, physical agents (radiation, heat, etc.), and medical devices.

Services include identification of hazardous chemicals and/or agents, assessment of exposure, evaluation of dose-response relationships, characterization of potential risk, identification of conditions adversely affecting health, design of health studies, and analysis and interpretation of data and scientific literature. Evaluations are both quantitative and qualitative in nature depending on the quality and quantity of available scientific data.

CONSULTING ACTIVITIES

The following are selected examples of work to evaluate and interpret:

- Airborne methylacrylate, isocyanate, and chlorine exposures and potential health effects
- Medical records and eyewitness accounts to determine cause of injury or death (traumatic, asphyxia, etc.) and related signs and symptoms of alleged toxic exposure
- Health effects of indoor exposures to carbon monoxide, chemicals, and microbial pathogens
- Potential exposures to toxic waste materials
- Alleged pesticide exposures
- Injuries from dermal and inhalational exposure to multiple solvents

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Sunnyvale, CA 94087-0299
Phone: 408-736-0446
Fax: 408-736-4033

CONSULTING ACTIVITIES (continued)

- Effects of carbon monoxide on health, neurological, and cognitive function
- Sewer gases and potential health risks
- Alleged exposure and adverse health effects of combustion products
- Lead exposures, blood lead concentrations, and neurological and cognitive outcome
- Potential interactions of calcium channel-blocking and beta-blocking drugs
- Bacterial contamination of intravenous drugs
- Health effects of medical devices including IUDs and breast implants
- Risk factors leading to diseases of various organs and organ systems

POSITIONS AND APPOINTMENTS

Susan A. Rice and Associates, Inc., Sunnyvale, CA
Toxicology and Pharmacology
President (1993-Present)

Failure Analysis Associates, Inc., Menlo Park, CA
Toxicology and Pharmacology
Senior/Managing Scientist (1990-1993)

SRI International, Menlo Park, CA
Biochemical Toxicology and Pharmacology
Senior Toxicologist, for conduct of NIH funded research (1990-1992)

Stanford University School of Medicine, Stanford, CA
Department of Anesthesia
Consulting Associate Professor of Pharmacology and Toxicology in Anesthesia (1990-Present)
Assistant/Associate Professor of Pharmacology and Toxicology in Anesthesia (1979-1990)
Postdoctoral Fellow/Research Associate in Anesthesia, Pharmacology and Toxicology (1976-1979)

Veterans Administration Medical Center (PAVAMC), Palo Alto, CA
Anesthesiology and Research Services
Associate Research Career Toxicologist/Pharmacologist (1987-1990)
Research Pharmacologist (1979-1987)
Postdoctoral Fellow/Research Associate in Anesthesia, Pharmacology and Toxicology (1976-1979)

School of Veterinary Medicine, University of California, Davis, CA
Department of Physiological Sciences
Walter Foster Fellow for Pulmonary Research (1974-1976)
Research Assistant/Laboratory Helper and Assistant I and II and Independent Study (1968-1974)

School of Public Health, University of California, Berkeley, CA
Department of Environmental Health Sciences
Assistant Specialist, Step II (1975-1976)

School of Medicine, University of California, Davis, CA
Department of Internal Medicine
Postgraduate Research Physiologist I (1971-1972)
California TB and Respiratory Disease Association Research Fellowship (1971)

PROFESSIONAL SOCIETY MEMBERSHIPS AND OFFICES

American Society of Anesthesiologists
American Society for Pharmacology and Experimental Therapeutics
California Society of Anesthesiologists
Genetic and Environmental Toxicology Association of Northern California
International Society for the Study of Xenobiotics
Neurobehavioral Teratology Society [formerly, Behavioral Teratology Society]
- President (1993-1994); President Elect (1992-1993)
- Secretary (1988-1992)
- Publications Committee (1990-1992; 1996-1999)
- Nominating Committee (1984-1985)

Northern California Chapter, Society of Toxicology
- Membership Committee (1991-1994), Chairperson (1991-1993)
- Nominating Committee (1986-1989)
- Treasurer (1995-1997)

Society for Neuroscience
Society of Toxicology
Teratology Society
Western Pharmacology Society
Western Teratology Society

VOLUNTARY SERVICE

California Environmental Protection Agency
- Comparative Risk Project for California, Human Health Subcommittee, Toxicology Dose-Response Work Group (1992-1994)

Stanford University School of Medicine
- Faculty Senate (1981-1989)
- Admissions Committee/Minority Admissions Committee (1979-1983)

Stanford University School of Medicine, Department of Anesthesia
- Committee on Resident Education (1980-1990)
- Committee on Medical Student Education (1983-1988)
  - Acting Chairperson (1984-1985)
- Committee on Biosafety (1979-1983)

Veterans Administration Medical Center
- Subcommittee on Safety for Medical Research Laboratories (1979-1990)
  - Chairperson (1989-1990)
- Occupational Health Consultant (1983-1990)
- Research and Development Subcommittee on Animal Studies (1983-1989)
  - Chairperson (1986-1989)

RESEARCH FUNDING

National Institute of General Medical Sciences # RO-1-22746
- PI - Nephrotoxicity of Fluorinated Anesthetics (1984-1985; 1985-1988; 1988-1992)
- Co-PI (Co-PI, R. Mazze) - Nephrotoxicity of Fluorinated Anesthetics (1982-1984)
- Investigator (PI, R. Mazze) - Nephrotoxicity of Fluorinated Anesthetics (1979-1982)
- Investigator (PI, R. Mazze) - Nephrotoxicity of Fluorinated Volatile Anesthetics (1977-1979)

RESEARCH FUNDING (continued)

National Institutes of Health, Biomedical Research Support Grant, Stanford University # 5353
PI - Characterization of Isoniazid-Induced Defluorinase Activity (1981-1982)

Stanford University School of Medicine, Department of Anesthesia Research Committee
PI - Behavioral Teratogenicity of Nitrous Oxide (1981)

Veterans Administration Merit Review Program
PI - Parental N_2O and Behavioral Effects and Mechanisms in Offspring (1987-1990)
PI - Behavioral Teratogenicity of Inhaled Anesthetic Agents (1981-1984; 1984-1987)
Investigator (PI, R. Mazze) - Anesthetic Toxicity and Metabolism (1977-1979; 1979-1983; 1983-1985)

University of California, Davis, Chancellor's Patent Fund Grant
PI - Pulmonary Toxicity of Thiocarbamides (1973-1974; 1974-1975; 1975-1976)

REVIEWER

Anesthesiology
Neurotoxicology and Teratology
(Editorial Board, 1990-1992; 1995-1998)
Fundamental and Applied Toxicology
Teratology
Toxicology and Applied Pharmacology

HONORS AND AWARDS

Diplomate of the American Board of Toxicology (1990-present)
VA Associate Research Career Scientist Award (Salary) resigned in 1990 (1987-1992)
Walter Foster Fellowship for Pulmonary Research (1974-1975; 1975-1976)
Chancellor's Patent Fund Grant, UC, Davis (1973-1974; 1974-1975; 1975-1976)
Summer, California Tuberculosis and Respiratory Disease Fellowship, UC, Davis (1971)

PUBLICATIONS

1. Giri SN and Rice SA: Influence of phenoxybenzamine on gastric evacuation in rats. Life Sciences 9:1109-1115, 1970.

2. Cronin [Rice] SR, Giri SN and Zimmerman RA: Aberrations in carbohydrate metabolism in response to pulmonary edematogenics. Proc West Pharmacol Soc 17:256-261, 1974.

3. Cronin [Rice] SR and Giri SN: Effects of pulmonary irritants on DNA, ATPase activity and histamine in rat lungs. Proc Soc Exp Biol Med 146:120-125, 1974.

4. Devereux DF, Rice SA and Giri SN: The effect of thermal burns on blood and plasma histamine levels in the rat and the cat. Proc West Pharmacol Soc 18:114-118, 1975.

5. Rice SA and Giri SN: Effect of thiourea on pulmonary vascular leakage in rats of different ages. Proc West Pharmacol Soc 18:128-132, 1975.

6. Giri SN, Benson J, Siegel DM, Rice SA and Schiedt M: Effects of pretreatment with anti-inflammatory drugs on ozone-induced lung damage in rats. Proc Soc Exp Biol Med 150:810-814, 1975.

7. Giri SN, Rice SA and Bacchetti P: Characteristic features of actinomycin D-induced paw inflammation of the rat. J Mol Path 23:367-378, 1975.

8. Devereux DF, Michas CA and Rice SA: Heparin pretreatment suppresses norepinephrine concentrations in dogs in endotoxic shock. Clin Chem 23:1346-1347, 1977.

9. Devereux DF, Rice SA and Giri SN: The effects of heparin pretreatment on plasma histamine following thermal injury in rats and cats. Circ Shock 5:311-316, 1978.

10. Baden JM, Kelley M, Simmon VF, Rice SA and Mazze RI: Fluroxene mutagenicity. Mutat Res 58:183-191, 1978.

11. Shu H, Talcott RE, Rice SA and Wei ET: Lipid peroxidation and paraquat toxicity. Biochem Pharmacol 28:327-331, 1979.

12. Dooley JR, Mazze RI, Rice SA and Borel JD: Is enflurane defluorination inducible in man? Anesthesiology 50:213-217, 1979.

13. Baden JM, Mazze RI, Wharton RS, Rice SA and Kosek JC: Carcinogenicity of halothane in Swiss/ICR mice. Anesthesiology 51:20-26, 1979.

14. Caughey GH, Rice SA, Kosek JC and Mazze RI: Effect of phenytoin (DPH) treatment on methoxyflurane metabolism in rats. J Pharmacol Exp Ther 210:180-185, 1979.

15. Wharton RS, Wilson AI, Mazze RI, Baden JM and Rice SA: Fetal morphology in mice exposed to halothane. Anesthesiology 51:532-537, 1979.

16. Rice SA and Talcott RE: Effects of isoniazid treatment on selected hepatic mixed function oxidases. Drug Metab Disp 7:260-262, 1979.

17. Sievenpiper TS, Rice SA, McClendon FD, Kosek JC and Mazze RI: Renal effects of enflurane anesthesia in Fischer 344 rats with pre-existing renal insufficiency. J Pharmacol Exp Ther 211:36-41, 1979.

18. Baden JM, Egbert B and Rice SA: Enflurane has no effect on haemopoiesis in mice. Br J Anaesth 52:471-474, 1980.

19. Baden JM, Rice SA, Wharton RS and Laughlin NK: Metabolic and toxicologic studies with enflurane in Swiss/ICR mice. J Environ Pathol Toxicol 4:293-303, 1980.

20. Fish K, Sievenpiper T, Rice SA, Wharton RS and Mazze RI: Renal function in Fischer 344 rats with chronic renal impairment after administration of enflurane and gentamicin. Anesthesiology 53:481-488, 1980.

21. Rice SA, Sbordone L and Mazze RI: Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following isoniazid administration. Anesthesiology 53:489-493, 1980.

22. Rice SA and Fish MP: Effects of Isoniazid Metabolites On the Rate of Hepatic Microsomal Defluorination of Volatile Fluorinated Ether Anesthetics. In: Microsomes, Drug Oxidations and Chemical Carcinogenesis. Vol II, 885-888. Coon MJ, et al. (Eds). Academic Press, 1980.

23. Baden JM, Rice SA and Mazze RI: Deuterated methoxyflurane anesthesia and renal function in Fischer 344 rats. Anesthesiology 56:203-206, 1982.

24. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in mice chronically exposed to nitrous oxide. Teratology 26:11-16, 1982.

25. Rice SA, Dooley JR and Mazze RI: Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following ethanol consumption. Anesthesiology 58:237-241, 1983.

26. Mazze RI, Rice SA, Wyrobek AJ, Felton JA, Brodsky JB and Baden JM: Germ cell studies in mice after prolonged exposure to nitrous oxide. Toxicol Appl Pharmacol 67:370-375, 1983.

27. Baden JM, Rice SA, Serra M, Kelley M and Mazze RI: Thymidine and methionine syntheses in pregnant rats exposed to nitrous oxide. Anesth Analg 62:738-741, 1983.

28. Fish KJ and Rice SA: Halothane inhibits metabolism of enflurane in Fischer 344 rats. Anesthesiology 59:417-420, 1983.

29. Rice SA, Sievenpiper TS and Mazze RI: Liver function and anesthetic metabolism in rats with chronic renal impairment. Anesthesiology 60:418-421, 1984.

30. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in rats exposed to nitrous oxide. Teratology 30:259-266, 1984.

31. Mazze RI, Rice SA and Baden JM: Halothane, isoflurane and enflurane MAC in mice and rats. Anesthesiology 62:339-341, 1985.

32. Rice SA, Mazze RI and Baden JM: Effects of subchronic intermittent exposure to nitrous oxide in Swiss Webster mice. J Environ Pathol Toxicol Oncology 6:271-281, 1985.

33. Mazze RI, Wilson AI and Rice SA: Fetal development in mice exposed to isoflurane. Teratology 32:339-345, 1985.

34. Rice SA, Baden JM and Kundomal YR: Effects of subchronic intermittent exposure to isoflurane in Swiss Webster mice. J Environ Pathol Toxicol Oncology 6:285-293, 1986.

35. Servin FF, Nivoche Y, Desmonts J, Rice SA and Mazze RI: Biotransformation of halothane and enflurane in patient with hyperthyroidism. Anesthesiology 64:387-391, 1986.

36. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden JM: Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. Anesthesiology 64:339-344, 1986.

37. Rice SA and Millan DP: Validation of a developmental swimming test using Swiss Webster mice treated perinatally with methimazole. Neurobehav Toxicol Teratol 8:69-75, 1986.

38. Rice SA and Fish KJ: Anesthetic metabolism and renal function in obese and non-obese Fischer 344 rats following enflurane or isoflurane anesthesia. Anesthesiology 65:28-34, 1986.

39. Wang S-L, Rice SA, Serra MT and Gross B: Purification and identification of rat cytosolic enzymes responsible for defluorination of methoxyflurane and fluoroacetate. Drug Metab Dispos 14:392-398, 1986.

40. Biermann JS, Rice SA, Gallagher E and West JA: Effect of diazepam treatment on hepatic microsomal anesthetic defluorinase activity. Arch Int Pharmacodyn Ther 283:181-192, 1986.

41. Rice SA, Maze M, Smith CM, Kosek JC and Mazze RI: Halothane hepatotoxicity in Fischer 344 rats pretreated with isoniazid. Toxicol Appl Pharmacol 87:411-419, 1987.

42. Rice SA, Millan DP and West JA: The behavioral effects of perinatal methimazole administration in Swiss Webster mice. Fund Appl Toxicol 8:531-540, 1987.

43. Fish KJ, Rice SA and Margary J: Contrasting effects of etomidate and propylene glycol upon enflurane metabolism and adrenal steroidogenesis in Fischer 344 rats. Anesthesiology 68:189-193, 1988.

44. Rice SA, Fish KJ, Hoover-Plow J, Jawaharlal K: In vitro hepatic drug and anesthetic metabolism of rats with dietary-induced obesity. Arch Int Pharmcodyn Ther, 299:286-293, 1989.

45. Biermann JS, Rice SA, Fish KJ and Serra MT: Metabolism of halothane in obese Fischer 344 rats. Anesthesiology 71:431-437, 1989.

46. Truog RD and Rice SA: Inorganic fluoride and prolonged isoflurane administration in he intensive care unit. Anesth Analg 69:843-845, 1989.

47. Pearl RG and Rice SA: Propylene glycol-induced pulmonary hypertension in sheep. Pharmacology 39:383-389, 1989.

48. Rice SA: Effect of prenatal N_2O exposure on startle reflex reactivity. Teratology 42:373-381, 1990.

49. Giri SN, Hollinger MA and Rice SA: Effects of thiourea on pulmonary vascular permeability and on lung and plasma histamine levels in rats. Toxicology Letters 57:283-290, 1991.

50. Giri SN, Hollinger MA and Rice SA: Effects of thiourea tolerance on plasma histamine, and lung vascular permeability. Archives of Toxicology 65:603-605, 1991.

51 Pearl RG, Baer ER, Siegel LC, Benson GV and Rice SA: Longitudinal distribution of pulmonary vascular resistance after endotoxin administration in sheep. Critical Care Medicine 20:119-125, 1992.

52. Hoover-Plow J and Rice S: Physical and behavioral development in two strains of mice fed excessive dietary vitamin B6. Nutrition Research 12:773-786, 1992.

53. Arnold JH, Troug RD and Rice SA: Prolonged administration of isoflurane to pediatric patients during mechanical ventilation. Anesth Analg 76:520-526, 1993.

ABSTRACTS

1. Mustafa MG, Cronin (Rice) SR, Duvall TR and Cross CE: Ozone effect on activities of lung cellular membrane enzymes. Clin Res 20:197, 1972.

2. Giri SN, Kartt LR, Joshi B and Cronin (Rice) SR: Mobilization of leucocytes and subsequent release of histamine and lysosomal enzymes into the peritoneal cavity and pleural effusions induced by the IP injection of actinomycin D. Fed Proc 33:558, 1974.

3. Giri SN, Kartt LR, Cronin (Rice) SR and Peoples SA: Inflammatory changes following subplantar administration of actinomycin D. Pharmacologist 16:292, 1974.

4. Rice SA and Giri SN: Histamine and pulmonary vascular permeability in tolerance to the lethal effects of thiourea. Pharmacologist 17:193, 1975.

5. Caughey G, Rice S, Hitt B and Mazze RI: Diphenylhydantoin (DPH) induction of methoxyflurane (MOF) metabolism in rats. Fed Proc 36:998, 1977.

6. Rice SA, Dooley JR and Mazze RI: Ethanol induction of hepatic microsomal defluorinase activity in rats. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, New Orleans, October 1977, pp. 349-350.

7. Baden JM, Kelley M, Mazze RI, Rice SA and Simmon VF: Fluroxene mutagenicity. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, New Orleans, October 1977, pp. 493-494.

8. Wharton RS, Sievenpiper TS, Wilson AI, Rice SA and Mazze RI: Developmental toxicity of methoxyflurane in mice. Teratology 17:41A, 1978.

9. Rice SA, Sbordone L and Mazze RI: Induction of in vitro anesthetic defluorination by isoniazid. Pharmacologist 20:258, 1978.

10. Sievenpiper TS, McClendon FD, Mazze RI and Rice SA: Renal effects and metabolism of enflurane in Fischer 344 rats with pre-existing renal insufficiency. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 303-304.

11. Wharton RS, Sievenpiper TS, Rice SA and Mazze RI: Developmental toxicity of methoxyflurane in Swiss/ICR mice. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 421-422.

12. Rice SA and Mazze RI: The induction of microsomal anesthetic defluorination by phenytoin. Abstract of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 571-572.

13. Rice SA, Sbordone L and Mazze RI: The induction of in vitro anesthetic defluorination by isoniazid. Abstracts of Scientific Papers, American Society of Anesthesiologists, Annual Meeting, Chicago, October 1978, pp. 573-574.

14. Rice SA, Maze M, Mazze RI and Kosek J: Hepatotoxicity following halothane anesthesia in isoniazid treated rats. Fed Proc 38:683, 1979.

15. Wharton RS, Wilson AI, Rice SA and Mazze RI: Teratogenicity of enflurane in Swiss/ICR mice. Teratology 19:53A, 1979.

16. Rice SA and Fish MP: Effects of isoniazid metabolites on the rate of hepatic microsomal defluorination of volatile florinated ether anesthetics. Abstract of Papers, Fourth International Symposium on Microsomes and Drug Oxidations, Ann Arbor, Michigan, July 1979, p. 111.

17. Rice SA, Baden JM and Mazze RI: Serum and urinary F^- levels following exposure to deuterated methoxyflurane (d4-MOF). Pharmacologist 21:214, 1979.

18. Fish MP and Rice SA: Isoniazid metabolites and anesthetic metabolism. Anesthesiology 51:S256, 1979.

19. Fish KJ, Sievenpiper TS, Rice SA and Mazze RI: Enflurane and gentamicin in chronic renal failure. Anesthesiology 51:S262, 1979.

20. Baden JM, Rice SA, Denson DD and Mazze RI: Deuterated methoxyflurane (d_4-MOF) Anesthesia. Anesthesiology 51:S263, 1979.

21. Rice SA and Mazze RI: Serum and urinary F^- levels and renal function following enflurane anesthesia in isoniazid treated rats. Fed Proc 39:998, 1980.

22. Baden JM, Rice SA and Mazze RI: Deuterated methoxyflurane anesthesia, No. 0028. World Conference on Clinical Pharmacology and Therapeutics, London, England, August 8, 1980.

23. Rice SA, Sievenpiper TS and Mazze RI: In vitro anesthetic defluorination and in vivo liver function in Fischer 344 rats with chronic renal failure. Anesthesiology 53:S253, 1980.

24. Rice SA and Mazze RI: Chronic intermittent exposure of Swiss W/ICR mice to N_2O. No. 457. Presented at Eighth International Congress of Pharmacology, IUPHAR, Tokyo, Japan, July 19-24, 1981.

25. Mazze RI, Rice SA, Baden JM and Wilson AI: Effects of nitrous oxide on fetal development and male fertility in mice. Anesthesiology 55:A188, 1981.

26. Rice SA: Developmental effects of N_2O exposure during organogenesis. Pharmacologist 24:163, 1982.

27. Rice SA, Mazze RI, Wyrobek AJ, Felton JS, Brodsky JB and Baden JM: Germ cell studies in mice after prolonged exposure to nitrous oxide. Anesthesiology 57:A368, 1982.

28. Fish KJ and Rice SA: Halothane inhibits metabolism of enflurane. Anesthesiology 57:A241, 1982.

29. Baden JM, Kelley M, Rice SA and Mazze RI: Decreased thymidine production in cells exposed to nitrous oxide. Anesthesiology 57:A243, 1982.

30. Rice SA, Millan DP and West J: The behavioral effects of perinatal methimazole exposure in Swiss Webster mice. Teratology 27:71A, 1983.

31. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in rats exposed to nitrous oxide. Anesthesiology 59:A373, 1983.

32. Mazze RI, Wilson AI, Rice SA and Baden JM: Effects of isoflurane on reproduction and fetal development in mice. Anesth Analg 63:249, 1984.

33. Rice SA, Mazze RI and Baden JM: Reproductive and teratogenic effects of isoflurane in Swiss Webster mice. Teratology 29:54A, 1984.

34. Rice SA: Behavioral effects of prenatal exposure to nitrous oxide (N_2O) in Swiss Webster mice. Teratology 29:7B, 1984.

35. Rice SA: Behavioral effects of prenatal exposure to nitrous oxide (N_2O) in Swiss Webster mice. Teratology No. 1797. Presented at the Ninth International Congress of Pharmacology, IUPHAR, London, England, July 1984.

36. Rice SA and Fish KJ: Comparison of the cytochrome P-450 mediated metabolism of fluorinated inhalation anesthetics and resultant nephrotoxicity in obese and normal male Fischer 344 rats. Presented at the Sixth International Symposium on Microsomes and Drug Oxidations, Brighton, England, August 5-10, 1984. Abstract No. P.3/53.

37. Rice SA and Fish KJ: Metabolism and renal function in non-obese and obese rats exposed to enflurane or isoflurane. Anesthesiology 61:A264, 1984.

38. Fish KJ, Rice SA and Weissman DB: Halothane inhibits metabolism of enflurane in surgical patients. Anesthesiology 61:A268, 1984.

39. Rice SA and Steffey EP: Metabolism of halothane and isoflurane in horses. Veterinary Surgery 14:76, 1985.

40. Serra MT and Rice SA: Relationship of isoniazid (INH) acetylation rate and defluorination rates of enflurane (ENF), isoflurane (ISO) and methoxyflurane (MOF). Fed Proc 44:1253, 1985.

41. Wang S-L, Rice SA and Serra MT: Identification of defluorination activity in purified rat liver cytosol glutathione S-transferases (GTs). Fed Proc 44:1821, 1985.

42. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden J: Reproductive effects of the inhalation anesthetics, halothane, enflurane, isoflurane and nitrous oxide. Teratology 31:55A, 1985.

43. Rice SA and Millan DP: Behavioral effects of in utero nitrous oxide (N_2O) exposure in adult SW mice. Teratology 31:6B, 1985.

44. Biermann JS, Rice SA and Fish KJ: Altered metabolism of halothane in obese Fischer 344 rats. Anesthesiology 63:A542, 1985.

45. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden JM: Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. Anesthesiology 63:A439, 1985.

46. Rice SA and Steffey EP: Metabolism of three inhaled anesthetics by cow, dog, lamb and rat hepatic microsomes. Proceedings, p. 164. Presented at the 2nd International Congress Veterinary Anesthesiologists, Sacramento, California, October 1985.

47. Rice SA: Prenatal N_2O exposure alters startle reflex in adult mice. Toxicologist 6:75, 1986.

48. Rice SA: Behavioral effects of in utero isoflurane exposure in young SW mice. Teratology 33:100C, 1986.

49. Margary J, Rice SA and Fish KJ: Propylene glycol and amidate^R inhibit enflurane metabolism in Fischer 344 rats. Anesthesiology 61:A249, 1986.

50. Rice SA and Wang S-L: Defluorination by hepatic glutathione S-transferases? Toxicologist 7:217, 1987.

51. Rice SA: Effects of prenatal isoflurane exposure on postweaning activity and reactivity in SW mice. Teratology 35:10B, 1987.

52. Rice SA: Effects of prenatal isoflurane exposure on postweaning activity and reactivity in SW mice. 10th International Congress of Pharmacology, IUPHAR, Sydney, Australia, August 1987.

53. Rice SA and Fish KJ: Anesthetic considerations for drug metabolism studies in laboratory animals. J Lab Animal Med 37:520, 1987.

54. Fish KJ, Rice SA and Margary J: Isoflurane metabolism in obese rats with chronic renal insufficiency. Anesthesiology 67:A303, 1987.

55. Baer ER, Pearl RG, Siegel LC and Rice SA: Longitudinal distribution of pulmonary vascular resistance during endotoxin-induced pulmonary hypertension in sheep. Anesthesiology 69:A860, 1988.

56. Pearl RG and Rice SA: Thromboxane mediation of propylene glycol-induced pulmonary hypertension in sheep. Anesthesiology 69:A861, 1988.

57. Rice SA and Ebling WF: F^- disposition in obese and non-obese F344 rats. Toxicologist 9:88, 1989.

58. Rice SA: Startle reflex reactivity of rats perinatally treated with methimazole or prenatally exposed to N_2O. Teratology, 39:508, 1989.

59. Rice SA and Carughi A: Embryonic and fetal folates following N_2O exposure. Toxicologist, 10:40, 1990.

60. Carughi A and Rice SA: Embryonic and fetal folates in N_2O-exposed rats. FASEB Journal, 4:A873, 1990.

61. Rice SA, Carughi A and Serra MT: Startle hyperreactivity following repeated prenatal N_2O exposure in SD rats. Teratology 41:619, 1990.

62. Fish KJ, Rice SA and Peterson KL: Renal function following cyclosporine and enflurane. Anesthesiology 73:A361, 1990.

63. Rice SA, Carughi A and Serra MT: Fetal brain catecholamines and indoleamines following repeated nitrous oxide exposure. Society for Neuroscience Abstracts 16:1118, 1990.

64. Rice SA, Phillips SJ, Allen KL, Gordon GR and Tyson CA: Acute effects of cyclosporin A and isoflurane anesthesia in a model of renal transplantation. Toxicologist 12:224, 1992.

65. Ray RM, Donelson A, Rice S and Kalinowski A: Fatal motor vehicle accidents: The role of drowsiness, fatigue & antihistamines with such effects. To be presented at the 12th World Congress of the International Association for Accident and Traffic Medicine, 7th Nordic Congress on Traffic Medicine, Helsinki, June 23-25, 1992.

BOOK CHAPTERS AND THESIS

1. Rice SA: The Role of Histamine in the Pathogenesis of Pulmonary Edema and in the Development of Tolerance to Thiocarbamide Compounds. Ph.D. Thesis, University of Michigan Microfilms, Ann Arbor, Michigan, 1977.

2. Baden JM and Rice SA: Metabolism and Toxicity of Inhaled Anesthetics. In: Anesthesia, Vol 1, pp. 383-424. Miller RD (Ed.). Churchill Livingstone Inc., New York, 1981.

3. Rice SA: Behavioral Toxicity of Inhalational Anaesthetic Agents. In: Clinics in Anaesthesiology, Vol 1, No 2, pp. 507-519. Mazze RI (Ed.). W.B. Saunders Company Ltd., London, 1983.

4. Rice SA and Pellegrini MJ: Basic Principles of Teratology. In: The Pregnant Surgical Patient, pp. 1-28. Baden JM and Brodsky JB (Eds.). Futura Publishing Company, Inc., New York, 1985.

5. Rice SA and Pellegrini MJ: Teratogenicity of Fixed Agents. In: The Pregnant Surgical Patient, pp. 53-82. Baden JM and Brodsky JB (Eds.). Futura Publishing Company, Inc., New York, 1985.

6. Baden JM and Rice SA: Metabolism and Toxicity of Inhaled Anesthetics. In: Anesthesia, Vol 1, pp. 701-744. Miller RD (Ed.). Churchill Livingstone Inc., New York, Second Edition, 1986.

7. Baden JM and Rice SA: Metabolism and Toxicity. In: Anesthesia, Vol 1, pp. 135-170. Miller, RD (Ed.). Churchill Livingstone Inc., New York, Third Edition, 1990.

8 Rice SA: Anaesthesia in Pregnancy and the Fetus: Toxicological Aspects. In: Effects on the Baby of Maternal Analgesia and Anaesthesia. pp 88-107. Reynolds F (Ed.). W.B. Saunders Company Limited, London, 1993.

9. Baden JM and Rice SA: Metabolism and Toxicity. In: Anesthesia, Vol 1, Chapter 8. pp 157-183. Miller, RD (Ed.). Churchill Livingstone Inc., New York, Fourth Edition, 1994 (in press).

10. Rice SA and Fish KJ: Anesthetic Toxicity: An Overview. In: Anesthetic Toxicity. pp 1-7. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

11. Rice SA: Reproductive and Develpomental Toxicity of Anesthetics in Animals. In: Anesthetic Toxicity. pp 157-174. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

12. Ebi K and Rice SA: Reproductive and Develpomental Toxicity of Anesthetics in Humans. In: Anesthetic Toxicity. pp 175-198. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

13. Rice SA: Developmental Neurotoxicity Tests - Data Evaluation. In: Handbook of Developmental Toxicity. Hood RD (Ed.). CRC Press, Inc., Boca Raton, Florida (in press).

BOOK

Rice SA and Fish KJ (Editors): Anesthetic Toxicity, Raven Press, Ltd., New York, 1994, 306 pp.

APPENDIX II

TABLES 1-5

Table 1: Comparison of Particulate Phases of Mainstream Smoke, Sidestream Smoke, and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Particle Number (no./cigarette)	10.5×10^{12}[b]	3.5×10^{12}[b]		U.S. DHHS, 1986
Particle Concentration (no. particles/cm^3)	5.3×10^9			U.S. DHHS, 1986
	10^9-10^{10}		~1.5×10^5	Rodgman, 1992
	~1×10^{10}			Guerin, 1987
Particle Concentration ($\mu g/m^3$)			18.4-64	U.S. EPA, 1992
			22.3 (7-77)[d,f]	Holcomb, 1993
			45.9 (ND-240)[d,g]	
			49.5 (17-212)[e,f]	
			67.7 (12-2700)[e,g]	
			103.7[e,h]	
			131.5 (ND-685)[e,i]	
			<120-986	IARC, 1986
			460[j,r]	Lofroth et al., 1989
			320-470[h,s]	
			>1500[k,n]	

Table 1 (con't): Comparison of Particulate Phases of Mainstream Smoke, Sidestream Smoke, and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Total Particle Weight (mg/cigarette)	15-40[p]	19.5-52[l]		U.S. DHHS, 1986
	15-40[b,c,p]	19.5-52[l,o,p]		
		16.9[m]		U.S. EPA, 1992
		16-36[n]		
		20-23[n]		
		19.8-23.4[q]		Guerin, 1987
Particle Size (μm)	0.1-1.0	0.01-0.80		U.S. DHHS, 1986 & NRC, 1986
Particle Mean Diameter (μm)	0.3-0.4[b]	0.2	0.15-0.20	Rodgman, 1992
	0.40[b]	0.32[b]		NRC, 1986 & U.S. DHHS, 1986

[a] Data from indicated sources
[b] Fresh & undiluted smoke
[c] Total particulate matter
[d] Nonsmoking area; ( ) = range
[e] Smoking area; ( ) = range
[f] Homes
[g] Office/public places
[h] Bars
[i] Restaurants
[j] Average
[k] Peak
[l] Calculated from SSS/MSS = 1.3
[m] Filtered 1R4F Kentucky Reference Cigarette
[n] Commercial cigarette
[o] Diluted SSS
[p] Unfiltered cigarette
[q] U.S. type cigarette blend
[r] 2R1 reference cigarette
[s] Total respirable particles

Table 2: Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Carbon monoxide (CO)[b]	mg/cig	mg/cig	mg/m^3	
			1.79-4.76	Lofroth et al., 1989
	10-23			IARC, 1986
	10-23			NRC, 1986
	14-23[e]	26.8-61		Hoffmann & Hecht, 1990
	10-23		25-108.1[d]	U.S. EPA, 1992
	54			
	11.3[g]	54.1	2.69	RJR, 1988
		49.6-58.1[i]		Guerin et al., 1987
			2.4[h]	Lofroth et al., 1989
			5.1-9.8[j]	
			3.4-6.9	Holcomb, 1992
			4.6	Guerin, 1992
Benzo[a]pyrene (BaP)	ηg/cig	ηg/cig	$ηg/m^3$	
	9.2[g]	147.9		RJR, 1988
			1.07	Guerin, 1992
	20-40		1.7-460	NRC, 1986
	20-40[e]	40-70	3.3-23.4	Hoffmann & Hecht, 1990
	20-40		50-140[k]	U.S. DHHS, 1986
			0.25-760	IARC, 1986
	5-78	25-199	2.8-760	IARC, 1983

Table 2 (con't): Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
4-(N-nitrosomethyl-amino)-1-(3-pyridyl)-1-butanone (NNK)	ηg/cig	ηg/cig	ηg/m^3	
			< 1-3	Guerin, 1992
		400	1.9-29.3	U.S. EPA, 1992
	80-770	190[f]		IARC, 1986
	84.0	419		RJR, 1988
	80-770[e]	200-1400		Hoffmann & Hecht, 1990
	100-1000	100-4000[n]		U.S. DHHS, 1986; NRC, 1986
N-nitrosonornicotine (NNN)	ηg/cig	ηg/cig	ηg/m^3	
			< 1-3	Guerin, 1992
			1.8-22.8	U.S. EPA, 1992
	120-3700	150-1700		Hoffmann & Hecht, 1990
	101[g]	171		RJR, 1988
	200-3000	150		IARC, 1986; **NRC, 1986**
N-nitrosodimethylamine (NDMA)	ηg/cig	ηg/cig	ηg/m^3	
			1.0-10	Guerin, 1992
	10-40		20-200	NRC, 1986
	ND[g,o]	298		RJR, 1988
	2-20	736	10-240	IARC, 1986
		200-800[n]		U.S. EPA, 1992
	0.1-180	200-1040	0-240	Hoffmann & Hecht, 1990

Table 2 (con't): Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
N-nitrosodiethylamine (NDEA)	ηg/cig	ηg/cig	ηg/m^3	
	ND-25		0-200	Hoffmann & Hecht, 1990
			< 10	Guerin, 1992
	ND			RJR, 1988
	ND-25	< 1000[p]		U.S. EPA, 1992
		200-1400		

[a] Data are complied from several sources
[b] 1 ppm = 1.15 mg/m3
[c] Cig = cigarette
[d] Calculated from SSS/MSS ratio = 2.5-4.7
[e] Unfiltered cigarette
[f] Filtered cigarette
[g] 1R4F reference cigarette
[h] 2R1 reference cigarette
[i] U.S. type blend cigarette
[j] Commercial cigarette
[k] Calculated from SSS/MSS ratio = 2.5-3.5
[m] Calculated from SSS/MSS ratio = 1-4
[n] Calculated from SSS/MSS ratio = 20-100
[o] Not detected
[p] Calculated from SSS/MSS ratio = < 40

Table 3: Species Differences in Minute Volume per Unit Lung Weight[a]

Species	minute volume (l/min)	lung weight (g)	minute volume/g lung (l/min-g)
mouse	0.021	0.20	0.105
rat	0.16	1.6	0.10
human (male)	6.4	1065.0	0.006

[a] Adapted from Table 6, Phalen (1984c)

Table 4: *In Vivo* Inhalation Studies of ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (F344)	M/F	4	10	5	90 d	Histopathology of: nasal cavity, larynx, trachea, lungs, liver, kidneys, adrenals, brain, pituitary, bladder, heart, thyroid, parathyroid, thymus, mammary gland, testes, ovaries	Hyperplasia & squamous metaplasia of epithelium of dorsal nasal turbinate of rats No significant effects in the other organs examined	von Meyerinck et al., 1989
					30 d recovery following 90 d exposure		Histopathological changes partially receded	
					60 d & 90 d recovery following 90 d exposure		Histopathological changes completely reversed	
Hamster (Syrian gold)	M/F				Same exposures as rats		No significant effects	
Rat (S/D)	M/F	0.1 1 10	6	5	14 d	Histopathology of: nasal passages, larynx, trachea, conducting airways, deep lung, heart, lymph nodes	Slight to mild epithelial hyperplasia & inflammation in rostral part of nasal cavity in 10 mg/m^3 group	Coggins et al., 1992
					14 d recovery following 14 d exposure		Histopathological changes were reversible	

Table 4 (con't): *In Vivo* Inhalation Studies of ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D)	M	0.1 1 10	6	5	4 d	Histopathology of: nasal passages, larynx, trachea, conducting airways, deep lung, heart, lymph nodes	Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	Coggins et al., 1993
					28 d		Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	
					90 d		Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	
					90 d recovery following 90 d exposure		No significant histopathological changes compared to control	
Rat (S/D)	M	2[a] 6[b]	7	7	90 d	Histopathology of: nose, larynx, vocal cords, trachea & lungs	Laryngeal epithelium: slight hyperplasia & slight squamous metaplasia Cuboidal epithelium: hyperplasia Pseudostratified epithelium: squamous metaplasia Vocal cord squamous epithelium: hyperplasia All histopathological changes occurred at 6 mg/m^3	Teredesai & Pruhs, 1994
					90 d	Thickness of: laryngeal epithelium and vocal cords	6 mg/m^3: +19% larynx & +32% lower medial surface of vocal cords; statistically different from control	
					21 d recovery following 90 d exposure	Histopathology of: nose, larynx, vocal cords, trachea & lungs	Histopathological changes were reversed except vocal cord hyperplasia	
Hamster (Syrian gold)	M				Same exposures as rats		No significant effects	

[a] (2 µg/L)
[b] (6 µg/L)

Table 5: *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Mouse (NMRI)	M	1 cig 2 cig 3 cig 4 cig	NA[c]	every 2 hr	NA	Clastogenesis @ 30 hr: micronuclei in femoral bone marrow polychromatic erythrocytes (PCE)	Clastogenesis (micronuclei/1000 cells): ~3.2 (1 cig); ~3.5 (2 cig); ~4.0 (3 cig); ~3.5 (4 cig) vs. ~1.75 (control) All significant increases from control	Mohtasham-ipur et al., 1987
Rat (S/D)	M & F	0.1 1 10	6	7	7 d	Chromosomal aberrations in alveolar macrophages	No significant differences from control	Lee et al., 1992
						DNA adduct formation[b]: lung, heart, larynx, liver	Lung (adducts/10^9 nucleotides): ~ 8.2 (10 mg/mg^3) vs. ~3.75 (control) Other organs & doses were not significantly different from control	
					14 d		Lung: ~8.7 (10 mg/mg^3) vs. ~3.75 (control) Heart: ~5.7 (10 mg/mg^3) vs. ~2.0 (control)	
					14 d recovery following 14 d exposure		Lung: ~8.0 (10 mg/mg^3) vs. ~3.75 (control) Heart: ~6.0 (10 mg/mg^3) vs. ~2.0 (control)	
Rat (S/D)	M	0.1 1 10	6	5	28 d	DNA adduct formation: lung, heart, larynx, liver & bladder	Lung (adducts/10^9 nucleotides): ~20 (10 mg/mg^3) vs. ~5 (control) Heart: ~7.5 (10 mg/mg^3) vs. ~3.0 (control) Larynx: ~10 (10 mg/mg^3) vs. ~4.5 (control) Liver: no significant differences from control Bladder: not measured	Lee et al., 1993

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D) (con't)	M	0.1 1 10	6	5	90 d		Lung: ~24 (10 mg/mg^3) vs. ~5 (control) Heart: ~17.5 (10 mg/mg^3) vs. ~5.0 (control) Larynx: ~27.5 (10 mg/mg^3) vs. ~4.5 (control Liver, bladder: not significantly different from control	Lee et al., 1993
					90 d recovery following 90 d exposure		Lung: ~15 (10 mg/mg^3) vs. ~6 (control) Heart: ~12.5 (10 mg/mg^3) group vs. ~4.5 (control) Larynx: ~13.0 (10 mg/mg^3) vs. ~5.0 (control) Liver, bladder: not significantly different from control	
Hamster (Syrian gold)	M	1.03	6	7 d	1 wk	Cell proliferation[c]: lung parenchyma, intrapulmonary airways, trachea, nasal passages	Nasal septum: cumulative labeling index 17.5% vs. 13% (control) Labeling indexes for all other sites were not significantly different from control	Witschi, et al., 1994
					1 wk recovery following 1 wk exposure		Terminal bronchioles: 11% vs. 8% (control) Maxillary turbinates: 14.5% vs. 20% (control)	
					2 wk exposure		Labeling indexes not significantly different from control	
					2 wk exposure followed by 1 wk recovery		Maxillary turbinates: 15% vs. 19.5% (control)	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Hamster (S. gold) (con't)	M	1.03	6	7 d	3 wk exposure		Labeling indexes not significantly different from control	Witschi, et al., 1994
					1 wk recovery following 3 wk exposure		Labeling indexes not significantly different from control	
Mouse (A/J)	M	1	6	5	1 d	Cell proliferation[c]: airways & lung parenchyma	No significant effects	Rajini et al., 1994
					3 d		Nasal epithelium of large airways: labeling index 8% vs. 3.5% (control) Terminal bronchioles: 7% vs. 2% (control)	
					5 d		Nasal epithelium of large airways: 11% vs. 5 % (control) Terminal bronchioles: 13% vs. 3% (control)	
Mouse (C57BL /6)	M	1	6	5	1 d 3 d 5 d	Cell proliferation[c]: airways & pulmonary parenchyma	No significant differences from control.	Rajini et al., 1994
Rat (S/D)	M	0.1 1 10	6	5	5 d	Cell proliferation[c]: nasal cavity, ventral larynx, trachea, & lung (bronchial, bronchioles, alveoli)	Cells labeled/1.5 mm nasal cuboidal: 88.5 in (1 mg/m^3), 168.1 (10 mg/m^3) vs. 40.5 (control) Cells labeled/1.0 mm nasal respiratory region: 26.1 (1 mg/m^3), 23.7 (10 mg/m^3) vs. 22.3 (control) Cells labeled/1.0 mm nasal squamous tissue: 299.5 (1 mg/m^3), 259 (10 mg/m) vs. 291.9 (control)	Ayres et al., 1995
					28 d		No significant differences from control	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D) (con't)	M	0.1 1 10	6	5	90 d	Cell proliferation[c]: nasal cavity, ventral larynx, trachea, lung (bronchial, bronchioles, alveoli)	Increased DNA synthesis in cuboidal & respiratory epithelium in most rostral portion of nasal cavity	Ayres et al., 1995
					90 d recovery following 90 d exposure		No increases in DNA synthesis in any tissue	
Mouse (A/J)	M	4	6	5	1 wk 2 wk 3 wk	Cell proliferation[c]: nasal cavity, trachea, lung	Few significant effects in lung Increase in cell proliferation in nasal passageways & somewhat in airways	Witschi et al., 1995
					4 wk		No significant effect	
					6 wk		No significant effects	
					9 wk		Some increase in cell proliferation in nasal & maxillary turbinates	
					16 wk		Some increase in cell proliferation in nasal & maxillary turbinates	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D)	neo-nate	0	NA	NA	birth	Lung P4501A1[d] & P4502B1[e]	No activity detected	Gebre-michael et al., 1995
		1.0	6	5	7 d		Lung P4501A1: detected in exposed group Lung P4502B1: detected, not different	
					14 d		Lung P450 1A1: ~9.5 pmol/mg/min (exposed) vs. 2.04 pmol/mg/min (control) Lung P4502B1: no significant differences	
					21 d		Lung P450 1A1: ~25 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
					50 d		Lung P450 1A1: ~37.5 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
					100 d		Lung P450 1A1: ~32.5 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
Rat (S/D)	M	1.0	6	4 d	4 d		Lung P4501A1 levels similar to those measured in 100 d exposure group Lung P4502B1: no significant differences	

[a] Not applicable
[b] P^{32} Post-labeling is used to quantify DNA adduct formation.
[c] Incorporation of BrdU is used to measure replicative DNA synthesis, a measure of cell proliferation.
[d] Ethoxyresorufin-O-dealkylase (EROD) activity is a measures of cytochrome P4501A1
[e] Pentoxyresorufin-O-dealkylase (PROD) activity is a measure of cytochrome P4502B1

COMMENTS ON

CHAPTER 7: *CARCINOGENIC EFFECTS OF EXPOSURE TO ENVIRONMENTAL TOBACCO SMOKE*

7.2 ETS AND CANCER SITES THAT ARE ASSOCIATED WITH ACTIVE SMOKING: LUNG CANCER

EXTERNAL REVIEW DRAFT: JANUARY, 1996

Submitted to

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Section

By

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1. EXECUTIVE SUMMARY

The U.S. EPA has, without regard to scientific method, made several basic--and incorrect--assumptions that fatally flaw Cal/EPA's ability to reach an unbiased, scientifically sound conclusion as to the relationship between environmental tobacco smoke (ETS) exposure and the risk of lung cancer. In accepting U.S. EPA's conclusion, Cal/EPA also accepts U.S. EPA's basic assumptions regarding ETS.

It is critical that Cal/EPA carefully assess all available and relevant toxicology data and make a thorough, unbiased, and scientifically-based assessment of the relationship or lack of relationship between exposure to ETS and lung cancer. Especially in light of the new information regarding the strength of the statistical associations in several of the epidemiology studies that Cal/EPA received during its workshop on March 25, 1996, it is imperative that the agency evaluate all available human and animal data relevant to the issue of ETS and lung cancer. In the case of ETS, the relevant toxicology data include a number of animal studies.

Pulmonary (bronchogenic) squamous cell carcinoma, the tumor type reported to be most closely associated with cigarette smoking in humans, has not been observed in either mice or hamsters exposed via inhalation to mainstream smoke, sidestream smoke, or ETS (Henry, 1986; IARC, 1986; Huber, 1989; Rodgman, 1992). Of all the inhalation studies performed, pulmonary squamous cell carcinoma has been observed in only one rat (F344) following exposure to cigarette smoke (Dalbey et al., 1980). The study was repeated two times (Heckman and Dalbey, 1982; Heckman and Lehman, 1985), but the initial observations were not confirmed.

The unique character of the complex mixture that is ETS makes extrapolation from mainstream smoke inappropriate. Contrary to the conclusions drawn by Cal/EPA based on

the epidemiology studies of ETS and the risk for lung cancer, the animal studies performed on aged and diluted sidestream smoke, the most appropriate surrogate for ETS, do not show and do not suggest an association between ETS exposure and lung cancer.

2. INTRODUCTION

Susan A. Rice and Associates, Inc. (SARA) is a consulting firm that specializes in the areas of toxicology and pharmacology. SARA has been asked to comment on Cal/EPA's external review draft by The R.J. Reynolds Tobacco Company. The comments provided herein are the result of review and analyses made by SARA, and the conclusions reached and the opinions expressed are those of SARA and do not necessarily reflect the conclusions or opinions of The R.J. Reynolds Tobacco Company. For the agency's information, the *curriculum vitae* and list of publications of Susan A. Rice, Ph.D., are included in Appendix I.

On March 25, 1996, the Office of Environmental Health Hazard Assessment (OEHHA) held a public workshop on environmental tobacco smoke (ETS) in order to elicit comments on its External Review Draft: *Carcinogenic Effects of Exposure to Environmental Tobacco Smoke: --Excerpt: ETS and Lung Cancer.* At that workshop, Richard A. Becker, Ph.D., Deputy Director for Scientific Affairs at OEHHA, stated that Cal/EPA intended to provide a scientifically-based assessment of ETS and the risk of lung Cancer. Furthermore, he stated that the agency was committed to including the best available information in its risk assessment.

The following information is provided to Cal/EPA to show that ETS is a unique, dilute, complex chemical mixture and to the point out some underlying assumptions that are currently a part of the review. The review draft document does not adequately scrutinize these assumptions. Specifically, it does not include a scientific assessment of the utility of

animal studies as an adjunct to the epidemiology studies, which have been used to evaluate the effects of ETS on the risk of lung cancer.

This commentary is not intended to be comprehensive. SARA recognizes that the limited discussions provide only an overview of selected aspects and do not adequately express the complexity of the subject matter. SARA acknowledges the level of energy and the depth of thought that Cal/EPA needs to invest to properly investigate the question of ETS and its potential to be a human pulmonary carcinogen. The basis for the assumption that ETS is a human pulmonary carcinogen needs to be critically evaluated, and science policy and health policy need to be clearly separated.

3. COMMENTS ON EXTERNAL DRAFT REVIEW

3.1. Draft Report Conclusions Based Primarily on Epidemiology Studies

Cal/EPA's conclusions presented in the external review draft inappropriately rest principally on the epidemiology studies related to ETS. The results of the epidemiology studies provide no consistent statistical evidence linking ETS exposure to the risk of lung cancer (e.g., Lee and Forey, 1995; Sugita et al., 1995; comments received at March 25, 1996, Cal/EPA workshop). Due to the very nature of epidemiology studies, there are many confounders that were not, and could not be, controlled even with the best of study designs.

Emphasis on the epidemiology studies completely ignores the fact that animal studies can augment the epidemiology studies, especially given the statistical weakness of the epidemiology studies, and advance the current scientific understanding of ETS, its potential to produce toxicity, and its relationship to mainstream smoke and to sidestream smoke. The draft review does not make one reference to or citation of an animal study.

3.2. Reliance on Analyses and Conclusions of U.S. EPA 1992 Report

Cal/EPA's draft report relies heavily on the analyses and conclusions of the U.S. EPA as contained in its 1992 report on ETS entitled *Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders* (U.S. EPA, 1992). Specifically, and without discussion or comment, Cal/EPA accepts U.S. EPA's conclusions that ETS is a human lung carcinogen and that there is compelling biologic plausibility of an effect of ETS exposure on the risk of lung cancer. Cal/EPA sought to support the conclusions of the U.S. EPA by stating that other earlier reports (IARC, 1986; NRC, 1986; U.S. DHHS, 1986) had reached similar conclusions.

In accepting the assumption that ETS is a human lung carcinogen, Cal/EPA has accepted the validity of U.S. EPA's evaluation of animal data. For several reasons that assumption is unwarranted and Cal/EPA's uncritical acceptance of that assumption demonstrates that the agency has not conducted an adequate review of the available data on ETS. First, the U.S. EPA examined only a selected subset of mainstream smoke and sidestream smoke exposure studies, and did not review all available relevant toxicology data. Second, U.S. EPA assumed that the observations made with mainstream smoke and sidestream smoke exposure studies were relevant to the evaluation of ETS and its relationship to lung cancer.

Propagating those assumptions will prevent Cal/EPA from conducting a scientifically valid analysis of the relationship, if any, between ETS and lung cancer. In order to claim a compelling biological plausibility, U.S. EPA had to assume that ETS is qualitatively the same as mainstream smoke, and that the observations made with mainstream and sidestream smoke studies are applicable to the evaluation of ETS exposure.

The U.S. EPA report (1992) did not consistently or adequately address the fact that ETS is a unique complex mixture that is different from both mainstream smoke and fresh

sidestream smoke (see sections 4.3 and 5.5 and Tables 1 and 2 in Appendix II). Because of inherent differences between the mixtures, observations made in mainstream smoke and sidestream smoke studies are not applicable to ETS. These differences include, for example, differences in particle sizes and particle retention in the lung, differences in the distribution of chemicals between the gas and particulate phases, and the extremely dilute nature of ETS compared with mainstream smoke and sidestream smoke (See Table 1). Dosimetrically, the difference between ETS exposure and cigarette smoking is immense. ETS is not dilute mainstream smoke, and any toxicological evaluation should respect that fact. Contrary to the U.S. EPA's assertion, a relationship between ETS exposure and lung cancer is not biologically plausible. The assumptions made by U.S. EPA (1992) do not conform to basic toxicologic principles. These assumptions fatally flawed the ability of U.S. EPA to reach an unbiased, scientifically sound conclusion as to the relationship between ETS exposure and the risk of lung cancer.

3.3. Information That Has Become Available Since Issuance of The 1992 U.S. EPA Report

Since the 1992 U.S. EPA report was written (U.S. EPA, 1992), additional relevant animal studies have been published that should be considered by Cal/EPA. New information has been developed regarding the mechanistic and toxicologic consequences of exposure to various concentrations of an ETS surrogate, aged and diluted sidestream smoke. These studies have examined toxicity and histopathology (Coggins et al., 1992, 1993; Teredesai and Pruhs, 1994), carcinogenicity (Witschi et al., 1995) and potential mechanisms of action such as cellular proliferation (Rajini et al., 1994; Ayres et al., 1995; Witschi et al., 1995), cytochrome P-450 (Gebremichael et al., 1995), DNA adduct formation (Lee et al., 1992, 1993), and chromosomal aberrations (Mohtashamipur et al., 1987; Lee et al., 1992).

Tables 2 and 3 (Appendix II) summarize the studies that have been performed in animals with an ETS surrogate, aged and diluted sidestream smoke (ADSS). The results of these studies show that at high particle concentrations of ADSS that is, ≥4 mg of particles per cubic meter (m^3), some hyperplastic changes were observed primarily in the nasal epithelium of rats. For perspective, human exposure to ETS, when it occurs, is typically less than 0.1 mg of total particulate matter/m^3; a significant portion of the particulates may not be ETS. Mice and hamsters do not exhibit these changes at the highest concentrations evaluated, 4 and 6 mg of particles/m^3, respectively. The observed hyperplastic changes in rats are fully reversible. These results suggest a reactive and adaptive response in the rats that is similar to responses in this animal model following exposure to other irritants. Studies of DNA adduct formation show increased adduct formation in ADSS-exposed rats only at a particle concentration of 10 mg/m^3. The lack of increased adduct formation at 1 mg/m^3 over a period of 90 days suggests that adduct formation at the considerably higher 10 mg/m^3 concentration is in response to overload of normal or usual metabolic pathways and/or defense mechanisms. Results of cellular proliferation studies are consistent with this interpretation. See section 6.2 for additional details.

4. Background Information in Consideration of Basic Principles

4.1 Scientific Method

The evaluation of the relationship of ETS to lung cancer should be examined in the spirit of the scientific method, which is the unbiased and controlled method of testing a hypothesis. A hypothesis is formulated, tested under well designed and properly controlled conditions, and is either confirmed or rejected on the basis of the results of observation and experimentation. The hypothesis is revised, if necessary, and verified by further observation and experimentation. Based upon all the available and relevant data, a theory is formulated that seeks to explain the process of interest.

In the case of the evaluation of ETS exposure and its relationship to lung cancer, the epidemiological evidence is not convincing and does not support a hypothesis of a causal role of ETS in lung cancer. If the hypothesis is not to be abandoned, then all remaining relevant data, which include the relevant animal studies, must be evaluated. This in turn will require scientific judgment as to which animal studies are relevant to the evaluation of ETS. The basis for this determination is consideration of the factors presented in sections 4.3 and 5.

4.2. Cause and Effect

Although an epidemiological association does not demonstrate cause and effect, many epidemiologists attempt to draw a causal inference (or judgment) based on epidemiological data. The propriety of such an approach is beyond the scope of this commentary, however, several criteria that are frequently considered in making a judgment on causation are similar for epidemiology and toxicology (Spilker, 1991). One of these criteria is the strength of the relationship or association between the exposure and the effect. For epidemiology the relative risk or odds ratio is used. The strength of the statistical relationship between ETS exposure and lung cancer in the epidemiology studies chosen by Cal/EPA is weak at best as was commented on by several presenters (i.e., W. Butler, G. Gori, M. Lavois, and M. Layard) at the Cal/EPA Workshop on March 25, 1996. A dose-response relationship is an important consideration in evaluating the strength of any relationship between an exposure and an effect. For toxicology, the strength of a relationship is represented by differences between control and test populations, trends in the data, and the statistical significance of that effect, usually at $p \leq 0.05$.

Of particular importance is the requirement that the effect be biologically plausible. The more specific the effect, the easier it should be to establish such a relationship. Difficulties arise when the observed effect is not specific to the chemical in question, but can be the

result of many independent or interdependent factors. There appears to be just such a problem with the study of ETS exposure. This is one of the many advantages of animal studies . Unlike epidemiological studies, animal studies are performed under controlled conditions, exposure can be quantified, and responses can be measured. An additional advantage of animal studies is that mechanisms of action can be investigated.

4.3. Composition of Mainstream Smoke, Sidestream Smoke, and ETS Complex Chemical Mixtures

Mainstream smoke is primarily ambient air drawn through cigarette paper and around a fire-cone that includes products of combustion (water and carbon dioxide) and products of incomplete combustion (organic constituents) (Guerin et al., 1987). The particulate matter is composed of 15-25% water and a wide variety of organic constituents. It is extremely concentrated (~1 x 10^{10} particles/cm^3 [Guerin et al., 1987]; 10.5 x 10^{12} particles/cigarette [U.S. DHHS, 1986]), and individual constituents are distributed between the particulate and vapor phases according to their solubility and volatility. See Tables 1 and 2 (Appendix II) for a comparison of selected constituents of mainstream smoke, sidestream smoke, and ETS.

Sidestream smoke differs from mainstream smoke in several ways. Sidestream smoke contains large quantities of vapor-phase water, and its higher alkalinity increases the proportion of nitrogen-containing compounds in sidestream smoke compared with mainstream smoke. The vapor-phase/particulate-phase distribution of the constituents is dependent on the degree to which sidestream smoke is diluted. Active smoking reduces the total sidestream smoke delivery, as should be expected. Fresh sidestream smoke contains 3.5 x 10^{12} particles/cigarette (U.S. DHHS, 1986) which is approximately one-third the number of particles in mainstream smoke. The particle size range of sidestream smoke is also decreased compared to mainstream smoke (see Table 1 in Appendix II).

The quantity and composition of the organic vapor phase is independent of the length of the cigarette remaining, which is in direct contrast to mainstream smoke, which becomes enriched in organic constituents with each puff. The vapor phase contains 90-95% of the nicotine in highly diluted sidestream smoke, and thus nicotine is not a good marker of particle deposition in the lungs.

ETS is comprised of aged and diluted exhaled mainstream smoke, sidestream smoke generated during the puff, and sidestream smoke generated during the smolder period between puffs. The greatest contributor to ETS is the latter. Together the sidestream smoke components contribute 85 to 90% of ETS. The particle concentration of ETS is dependent on its dilution in ambient air, and is orders of magnitude less than that of mainstream smoke (i.e., ~1.5 x 10^5 particles/cm^3 vs. ~10^{10} particles/cm^3) (Guerin et al., 1987; Rodgman, 1992). Smoking an unfiltered cigarette, a smoker inhales 15-40 mg of particles (U.S. EPA, 1992). Assuming a 50% deposition in the lung, the smoker retains ≥ 7.5 mg of mainstream smoke particles/cigarette.

In an ETS environment, a nonsmoker is usually exposed to significantly less than 0.1 mg of ETS particles/m^3 (see Table 1, Appendix II) and would inhale 0.024-0.24 mg of ETS particles in 8 hours (Scherer et al., 1990). An 11% retention of particles would result in a daily dose of ≤ 26.4 μg/day. Holcomb (1993) performed calculations based on an extensive review of indoor ETS concentrations and exposures. He estimates that maximum exposure of an adult male to ETS results in retention of 108.7 μg of ETS particles/day. A smoker by comparison retains ≥ 69 times that amount with each cigarette smoked or ≥ 1380 times that per day for 20 cigarettes smoked.

There are many factors in addition to deposition that contribute to the dose that the pulmonary tissues receive. These additional factors, only a few of which are mentioned in section 5.5, would further significantly decrease the total dose of ETS particulate matter in

the lungs of nonsmokers relative to the dose of mainstream particulate matter that smokers would receive. Gori and Mantel (1991) estimate that the actual tissue dose to a nonsmoker exposed to ETS may be less than 1/10,000 of the tissue dose that is received by a smoker.

5. ISSUES FOR CONSIDERATION

5.1. Utility of Animal Studies in Evaluation of Carcinogenicity and Determination of Mechanisms of Action

There are a variety of confounding factors in epidemiology studies, such as diet and lifestyle, in addition to the great underlying genetic variability. All of these may be associated with an observed effect independent of the chemical exposure under investigation.

Animal studies offer the opportunity to control conditions that were not controlled effectively or consistently in the epidemiological studies of ETS, and to identify what if any effects are produced at a range of concentrations that more appropriately represents the human exposure to ETS. Animal studies can provide information that is not readily available from epidemiology studies, such as measurements of cellular proliferation, chromosomal aberrations, DNA adduct formation, and evaluation of histopathology.

There has been some discussion of the utility of animals for the study of the carcinogenic effects of cigarette smoking, generally because of inconsistency in identifying in rodents significant increases in the numbers and kinds of tumors that have been associated with cigarette smoking in humans (Henry and Kouri, 1986; IARC, 1986). Pulmonary (bronchogenic) squamous cell carcinoma, the tumor type reported to be most closely associated with cigarette smoking in humans, has not been observed in either mice or hamsters exposed via inhalation to mainstream smoke, sidestream smoke, or ETS (Henry and Kouri, 1986; IARC, 1986; Huber, 1989; Rodgman, 1992). Of all the inhalation studies

performed, pulmonary squamous cell carcinoma has been observed in only one rat (F344) following exposure to cigarette smoke (Dalbey et al., 1980). The study was repeated two times (Heckman and Dalbey, 1982; Heckman and Lehman, 1985), but the initial observations were not confirmed.

In spite of the relative lack of tumorigenic response of rodents to high concentrations of cigarette smoke, rodents may in fact be appropriate models of the carcinogenic potential of cigarette smoke and ETS. Factors in favor of using certain strains of mice for the study of cigarette smoke (Henry and Kouri, 1986) and ETS or its surrogate, include these:

- Lung aryl hydroxylases are induced in response to smoke exposure
- Sister chromatid exchanges in bone marrow cells are increased following smoke exposure
- DNA repair capacity is inhibited approximately 50% in the lungs of smoke-exposed mice
- DNA synthesis following smoke exposure is increased up to twenty fold
- High incidences of squamous cell carcinoma can be produced with known chemical carcinogens

5.2. Mechanisms of Carcinogenesis

The mechanisms of carcinogenesis are not well understood. There are a number of hypotheses to explain various aspects of carcinogensis, but there is much about this complex process that is unknown. There are so many influences on the process that a carcinogen must be defined with respect to species, age, dose, route and frequency of administration, age, and other factors.

Chemical carcinogenesis is the complex, multistage process in which a chemical or its metabolite disrupts normal cell growth and regulation. An understanding of this process is

important when evaluating the carcinogenic potential of a chemical mixture such as ETS, because carcinogenesis is not a simple "one-hit" process. Exposure to a mixture that contains a "carcinogenic" chemical does not guarantee that cancer will develop.

The multistep nature of carcinogenesis has been explained by a number of researchers in terms of stages: initiation, promotion, and progression. Multiple steps may in turn be present within each stage (Trosko and Chang, 1988; IARC, 1992; Barrett, 1993). Three of the processes currently recognized to be important in chemically-induced initiation of carcinogenesis are: metabolism to a reactive chemical species, DNA repair, and cell proliferation (Ames et al., 1993). Mutations, for example, transitions, transversions, and deletions, occur as a result of endogenous metabolic processes, radiation, and exogenous chemicals. Chemical initiators interact with DNA either directly or indirectly through an active metabolite to effect the above changes. Initiation is irreversible because the mutation becomes "fixed" or permanent as a result of DNA synthesis and cell division (mitosis); that is, the genotype and/or phenotype of the initiated cell is established. At this stage in carcinogenesis, any change may be subtle; neoplasia may not result because of apoptosis (programmed cell death) or because promotion and/or progression do not follow initiation (Pitot and Dragan, 1996).

Promotion is a reversible process that inhibits apoptosis and/or enhances or represses gene expression. Gene expression is altered primarily through perturbation of signal transduction pathways (Pitot and Dragan, 1996). One hypothesis is that promoting agents act through specific receptors and that the promoter's effect is directly proportional to the number of receptors that it occupies.

Another hypothesis is that promoting agents selectively increase proliferation and may also decrease apoptosis of preneoplastic cell populations. The process of promotion is subject to modulation by many factors such as diet, hormones, and age. Although understanding of

cell cycle regulation has increased dramatically in the past five years, there is still much about the process of promotion that is unknown. A promoter cannot induce cancer in and of itself; its role in carcinogenesis is dependent on an initiated population of cells.

Progression is the third stage of carcinogenesis in which complex genetic alterations occur as a result of evolving karyotypic instability. Cell proliferation has been linked to the carcinogenic process, and many chemicals that are cytotoxic at high concentrations induce regenerative cell proliferation (IARC, 1992). Increased cell proliferation can saturate the DNA repair mechanisms that correct mutations induced by normal endogenous reactive oxygen species formed during metabolism, such as super oxide anion, and peroxides. Mutations may thus be a secondary result of cellular proliferation. In addition to cellular proliferation and secondary mutagenesis, cytotoxicants may induce inflammation or may increase the levels of circulating growth factors which may preferentially increase the growth of preneoplastic cells (Butterworth et al., 1995). The presence of cell proliferation is not sufficient of itself to produce a tumor.

During this stage of carcinogenesis, additional changes occur in the genome that alter cell growth rate and responses to hormonal influences (Trosko and Chang, 1988; Pitot and Dragan, 1996). As stated by Pitot and Dragan (1996),

> "very low doses of complete carcinogens act to initiate cells but cannot sustain the remainder of the carcinogenic process. This consideration is undoubtedly very important in carcinogenesis in humans, in whom most exposures are at extremely or relatively low levels of a carcinogenic agent."

Current understanding of carcinogenesis recognizes that the probability of a chemical producing a cancer is dependent on many factors including the cell type, the site and type of chemical action within that cell, the repair capabilities of the cell, and the rate of

mitogenesis and apoptosis. Experimental studies have established that different chemicals act at various stages in the carcinogenic process. It is inappropriate to extrapolate the effects observed from a high dose to a low dose because different mechanisms of action may be active at different dose levels. See section 5.3 for further discussion.

5.3. Dose-Response Relationships

5.3.1. Relationship of Dose to the Process of Carcinogenesis

A threshold dose is a dose above which a clear response is observed or, alternatively, a dose below which no effects of interest are observed. This concept is widely accepted for noncancer toxicity endpoints, but it has been the subject of some controversy for cancer (Zeise et al., 1987; Upton, 1988; Beck et al., 1994; Sagan, 1994; Cohen, 1995; Hrudey and Krewski, 1995; Purchase and Auton, 1995). There are many factors that contribute to the ultimate expression of toxicity or disease, and disruption of any of the intermediate steps in that process can affect the observed threshold. Threshold can be explained by many factors, including the chemical failing to reach its target site or to attain a significant concentration at the target site (molecular dose). The natural capacity of a cell to repair itself also influences threshold.

A widely quoted review of dose-response relationships published by Zeise et al. (1987) was extensively used by Purchase and Auton (1995) for their discussion of thresholds in chemical carcinogenesis. They stated:

> "In the field of chemical carcinogenesis, there is a growing body of evidence to suggest that there are mechanisms of cancer induction which display the type of mechanistic threshold observed in other types of toxicity. Nongenotoxic carcinogens act by mechanisms which do not involve the direct interaction of the chemical or its metabolites with DNA."

An example they presented is the production of thyroid cancer by chemical carcinogens that interfere with thyroxin homeostasis and result in an excess of thyroid-stimulating hormone, which results in hyperplasia and eventually cancer of the thyroid. Purchase and Auton (1995) also stated that it is difficult to identify thresholds in epidemiology studies even when the dose-response relationship is sublinear. Likewise, in animal studies even an apparent threshold at low dose cannot be proven statistically because it is equivalent to proving a negative.

According to Purchase and Auton (1995), "The selection of the method of risk assessment on the basis of the presence or absence of a threshold can only be justified by consideration of mechanistic information of the toxicity under study." The effects of carcinogenic agents at very low doses become not only indistinguishable from the background incidence, but they appear insignificant when set in the context of the risks associated with other accepted societal activities.

5.3.2. High-Dose Extrapolation

The effects that are observed following high-dose exposure to chemicals or chemical mixtures are not necessarily representative of the effects that would be seen following lower doses, because the mechanisms of action leading to the observed effects at high doses may not be in action at lower doses. This is especially true for chemicals that are cytotoxic or mitogenic at high doses. In addition, normal metabolic pathways can be overwhelmed when confronted with massive amounts of a chemical. The concept of metabolic overload and its consequences have been discussed in various ways by a number of authors (Faccini et al., 1992; Wynder and Williams, 1992; Sagan, 1994). When overload occurs, the chemical concentration in the body continues to increase because the excretion pathways are saturated and/or new pathways that do not normally handle this type of chemical become involved. When new metabolic pathways are utilized, toxic metabolites

may be formed. In addition, the chemical may interact with some other macromolecule, such as a protein, or an endogenous sulfhydryl, such as glutathione. The interaction with the protein may be directly toxic by inactivating an enzyme; the interaction with glutathione may not be toxic in an of itself, but it may deplete the cell of this protective molecule and thus prime the cell for future injury by another, or more of the same, chemical.

Thus, at high doses there may be mechanisms in action that are not at all reflective of the mechanisms of chemical action at lower doses. Consequently, observations made at high doses cannot be directly extrapolated to lower doses.

Even if ETS were dilute mainstream smoke, which it is not, mainstream smoke studies are inappropriate to determine the likely effects of ETS simply because of significant differences in dose. In the case of ETS, there are additional reasons to reject observations made with high-dose sidestream smoke and mainstream smoke studies because the mixtures are also different from ETS in the concentration and distribution of their relative components.

5.3.2. Adaptive Repair

Recent discussion about the ability of an organism to respond to an insult has focused on whether a response should be described as a "toxic" response in all cases or whether the response could better be described as an "adaptive" response (Burger et al., 1989; Calabrese, 1992; Farber, 1992; Sagan, 1994). Burger et al. (1989) have addressed this question for laryngeal squamous cell metaplasia, changes in goblet cells of the nasal epithelium, macrophage accumulation within alveoli, and bronchiolization of the alveolar epithelium. In their review they have summarized the observations and conclusions of several pathologists and have come to the conclusion that the above-mentioned responses can indeed be adaptive responses that are not preneoplastic. Several authors have noted

that exposure to low levels of radiation or chemicals can produce adaptive effects that are protective against exposure at higher levels (Calabrese, 1992; Farber, 1992; Sagan, 1994).

5.3.3. Other Sources of Mutagens and Animal Carcinogens Reported to be in Mainstream Smoke, Sidestream Smoke, or ETS

Mutagens and animal carcinogens reported to be present in mainstream smoke, sidestream smoke, or ETS are reported to have many sources, with the possible exception of what have been called the tobacco-specific nitrosamines. Humans are routinely exposed to chemicals, principally via inhalation of airborne material; ingestion of food, beverages, and water; and dermal absorption of handled materials, soils, and radiation. Several approaches have been used to assess human exposure, including the U.S. EPA's Total Assessment Exposure Assessment Methodology (TEAM), which used direct measurement for multimedia studies (Waldman et al., 1991) and the Total Human Environmental Exposure Study (THEES) (Waldman et al., 1991). These studies will not be discussed here, but benzo[a]pyrene will be used as an example of a pollutant present in everyday life.

The class of compounds known as polyaromatic hydrocarbons (PAHs) are of interest because of their reported presence in mainstream smoke and ETS. Benzo[a]pyrene was the PAH chosen for measurement in THEES (Waldman et al., 1991). This study identified that in outdoor air the ambient concentrations of benzo[a]pyrene strongly influenced total inhalation exposure. Additionally, cooking activities, combustion appliances, and cigarette smoke were named as important sources of indoor air exposures. Dietary exposure to benzo[a]pyrene in this study ranged from 2 to 500 ηg per day, which was greater than daily inhalation of 10 to 50 ηg per day. Hattemer-Frey and Travis (1991) state that,

> "the food chain is the dominant pathway of human exposure, accounting for about 97% of the total daily intake of BaP [benzo[a]pyrene]. Inhalation and consumption

> of contaminated water are only minor pathways of human exposure. The long-term average daily intake of benzo[a]pyrene by the general population of the U.S. is estimated to be 2.2 micrograms (μg) per day. Cigarette smoking and indoor activities do not substantially increase human exposure to BaP relative to exposures to background levels of BaP present in the environment."

The heating of certain foodstuffs, such as broiled meat and fish, are known to significantly increase the PAH content of the food as well as the indoor air (Waldman et al., 1991). A single 8-ounce serving of charcoal-broiled T-bone steak could provide 420 to 716 mg of benzo[a]pyrene which is ~ 9 to 143 times the amount in the mainstream smoke from one cigarette and up to over 700 times the amount that would be inhaled in ETS over an 8-hour period (IARC, 1983).

5.5. Inhalation, Deposition, and Absorption of Materials

There are many factors that influence the total dose that the lung will experience. Some of these factors are species, age, sex, respiratory rate, tidal volume, mucociliary clearance, permeability of the alveolar-capillary carrier, activity and number of pulmonary macrophages, etc. A few factors from this list are discussed below as examples of the differences between the smoker and the nonsmoker. Not only are cigarette smoking and ETS inhalation extremely different activities and mainstream smoke and ETS very different complex mixtures, the smoker and the nonsmoker are themselves physiologically quite different.

5.5.1. Retention of Mainstream Smoke and ETS Particles

In determining the potential toxicity of an agent, it is important to evaluate the total amount of chemical that is delivered to an animal and the dose that is actually retained and ultimately absorbed. Differences between ETS and mainstream smoke in their deposition

and retention provide another reason for not extrapolating the effects of one to the other. For active smokers the percentage of particles from mainstream smoke that is deposited in the lung reportedly ranges between 50 to 90% (Gori and Mantel, 1991; U.S. EPA, 1992). In contrast, approximately 11% (Hiller, 1984) to 43% (McAughey et al., 1994) of the particles from ETS are deposited in the lungs of nonsmokers. Because the number of particles in mainstream smoke is significantly higher than in ETS, the active smoker receives a much greater unit dose and daily dose of particles and their associated chemicals than the nonsmoker does. Thus, studies of mainstream smoke are not studies of ETS and the observations made in these studies cannot be extrapolated to ETS.

5.5.2 Activity of Clearance Mechanisms

There are three main ways in which materials deposited in the lung are removed: mucociliary transport up the airway tree; dissolution into ions, atoms, or molecules that are transported in the blood; and drainage to lung-associated lymph nodes (Valberg and Blanchard, 1991). Clearance from the lung interstitium is via translocation to the lung lymph nodes, which in turn are cleared very slowly. For "insoluble" materials, the mucociliary pathway is most active 0 to 48 hours after exposure. It clears particles on the mucus lining of the ciliated airways. Smaller animal species have slower velocities of clearance than larger species. This being true, one would expect that data collected from rodent exposure might overestimate human toxicity.

The alveolar macrophage is another means for the lung to clear debris. Once particles are engulfed, macrophages are then cleared via transport up the mucociliary pathway. The calculated number of alveolar macrophages per alveolus is 0.037 for mice, 0.11 for rats, and 6.8 for humans; or, put another way, the area patrolled by each alveolar macrophage is 190,000, 140,000, and 22,000 μm^2, respectively (Valberg and Blanchard, 1991). From the

numbers, one can surmise that alveolar macrophages will not play as prominent a role in the clearance of particles from the lungs of rodents as from the lungs of humans.

5.5.3. Instillation Studies in Animals

Some of the most significant effects that have been produced related to mainstream and sidestream smoke are related to the instillation or implantation of concentrated forms of the two smokes (Wynder and Hoffmann, 1967). In some studies, reported animal carcinogens that are components of mainstream smoke have been instilled at very high concentrations into the lungs of animals (IARC, 1986). Instillation of many chemicals is known to produce an inflammatory reaction (Valberg and Blanchard, 1991). Aviado (1995) comments that "the animal model has lost defensive mechanisms to prevent absorption in the bronchopulmonary system" which are normally present and protect against inhaled chemicals. More importantly from a mechanistic point of view, instillation also has been observed to increase epithelial mitotic rates and to enhance hamster respiratory carcinogenesis (Valberg and Blanchard, 1991). The increased mitotic rates may be responsible for preventing normal DNA repair and for fixing mutations.

It is not surprising in the case of either instillation or implantation that the incidence of tumors is significantly increased over the control rates. In light of the earlier discussion of the mechanisms of chemical action and the process of carcinogenesis in section 5.2, it is obvious that the results of these studies are, first, inappropriate to evaluate the carcinogenic potential of mainstream and sidestream smoke, and, second, irrelevant for the evaluation of the carcinogenic potential of ETS.

5.6. Relationship of Particle Size and Toxicity

Cells in the respiratory tract are larger than mainstream smoke, sidestream smoke, or ETS particles that might settle in the respiratory tract. Adjacent cells may receive very different

doses depending on the deposition of particles on individual cells. The size of the particle that is deposited may have a significant effect on toxicity because the mass of a spherical particle is proportional to the cube of the geometric diameter. One thousand (1000) particles of 0.1 μm diameter must be deposited in the lung to equal the mass burden from the deposition of a single 1-μm diameter particle (Phalen, 1984a). The size of the particle may affect its clearance from the lung by mucociliary mechanisms or macrophages or its dissolution in the surrounding fluid. The effect on toxicity may be significant.

5.7. Knowledge of Individual Components Has No Predictive Value for Evaluating a Complex Chemical Mixtures

The effects of a complex chemical mixtures cannot be predicted from knowledge of the effects of its individual components. Interactive effects can be derived, for example, from biologic interactions within the animal or human and from physical and chemical interactions in the air, including adsorption of gases on particle surfaces, hygroscopicity, changes in particle size, etc. (Phalen, 1984b). Other factors may influence the total dose and the sites of deposition and absorbance. Irritancy and acidity are examples of factors that may influence the rate of breathing and other processes. Interactions of the individual components of a complex mixture at a cellular level may produce biologic effects that are synergistic, additive, or antagonistic. Thus, knowledge of the effects of individual components is not predictive of the effects of the mixture. The only way to evaluate the potential effects of the complex mixture known as ETS is to test ETS.

The mouse and rat have relatively higher minute volumes per gram of lung than do humans (See Table 4 in Appendix II). These rodents can potentially receive much higher doses of an inhaled chemical mixture per unit of lung weight than humans. This is important when one considers the predictability of toxicity from one species to another. In the case of ETS, proportionately the mouse and the rat could potentially receive approximately 16 times the

inhaled dose that a human male would receive (0.105/0.006 = ~16). Of course, many other factors are operative, such as the differences in the nasal pharyngeal anatomy between rodents and humans. The primary disadvantage in inhalation studies with rats and mice, in fact, derives from their short, relatively wide airways and their tendency to lack respiratory bronchioles (Phalen, 1984c). In spite of this shortcoming, the fact that mice and rats are obligate nose-breathers, and the fact that their nasal turbinates are significantly different from humans', there is no reason to suspect that either a mouse or a rat would receive less of a dose to the lung than would a human.

In addition to the animal's breathing pattern and the properties of an inhaled material, the anatomy of the respiratory system airspaces will determine how much of a pollutant will initially deposit within the subject and where it will be deposited. Typically, the mechanisms of diffusion, sedimentation, and impaction are important in particle deposition. (Phalen, 1984c)

6. Evaluation of Animal Studies Relevant to ETS

6.1. Aged and Diluted Sidestream Smoke (ADSS) as a Surrogate for ETS

Although it is preferable to study ETS directly, there are practical limitations that arise from its dilute nature. For this reason, aged and diluted sidestream smoke was developed as a surrogate for ETS. ADSS only lacks the exhaled mainstream smoke component and, thus, is the best surrogate for ETS. Aviado (1995) states, "This author, after review of animal studies, has concluded that an inhalation technique using aged and diluted SSS [sidestream smoke] is the only acceptable procedure by which to obtain data that are relevant to a claim that ETS is a pulmonary carcinogen." Cal/EPA should give special attention to those animal studies that have been performed utilizing aged, diluted

sidestream smoke as a surrogate for ETS because they provide the best available means for the evaluation of ETS toxicity at concentrations that are relevant for human exposure.

6.2. Summary of Observations Made in Studies with ADSS

Of publications in which ADSS was used, 11 were identified as being relevant for the evaluation of ETS pulmonary toxicity and carcinogenicity. Tables 4 and 5 (Appendix II) summarize the concentrations, evaluated endpoints, and results of these studies. Additional information may be found in the abstract for each article (included in section 6.3). The original articles for these studies are included in Appendix III.

For ease of evaluation, the studies have been divided into two tables, one of toxicology studies and the other of mechanistic studies. A single publication may be represented in both tables.

6.2.1 Inhalation Studies of Toxicity and Carcinogenicity

In two studies, inhalation of high concentrations of ADSS (i.e., 4 and 10 mg/m^3) has been shown only to produce reversible, slight to mild epithelial hyperplasia and inflammation of the nasal cavity. Von Meyerinck et al. (1989) exposed rats and hamsters to 4 mg/m^3 of ADSS for 10 hours a day, 5 days a week for 90 days. Coggins et al. (1993) exposed rats to 0.1, 1.0, or 10 mg/m^3 of ADSS for 6 hours a day, 5 days a week for 90 days.

The recovery phase that was designed in each study provides significant information relevant to the mechanism of toxicity and the potential for carcinogenicity. The reversibility of effects at the extremely high particle concentrations of 4 and 10 mg/m^3 strongly suggests that the observed changes were reactive and adaptive responses to repeated irritation rather than toxic responses *per se* (Burger et al., 1989).

No histopathological changes were observed by von Meyerinck et al. (1989) in ADSS exposed hamsters. Especially in light of a lack of effects in hamsters and a reversible effect in rats, it is indeed unfortunate that U.S. EPA omitted the work of von Meyerinck et al. (1989) from its 1992 report.

The findings of these investigators (von Meyerinck et al., 1989; Coggins et al., 1993) are further supported by a recently published study by Witschi et al. (1995). A/J mice were exposed by inhalation for a 6-month period to 4 mg/m^3 of ADSS for 6 hours per day, 5 days per week. Researchers in that study observed no effects of ETS exposure on tumor incidence. These observations are significant and interesting because the A/J mouse strain is known to be a sensitive test system that exhibits tumors within 6 months in response to a number of carcinogens.

6.2.2 Inhalation Studies of Cellular Proliferation

The results of cellular proliferation studies performed in mice by Rajini et al. (1994) and Witschi et al. (1994, 1995) and in rats by Ayres et al. (1995) are consistent with the above histopathological observations. Results of the studies of cellular proliferation are consistent with the presence of reactive and adaptive tissue responses. Similar to the observed histopathological changes, the proliferative changes are reversible.

6.2.3. Inhalation Studies of DNA Adduct Formation

Studies of DNA adduct formation show increased adduct formation in ADSS-exposed rats (Lee et al., 1992, 1993) only at a particle concentration of 10 mg/m^3. The lack of increased adduct formation at 1 mg/m^3 over a period of 90 days suggests that adduct formation at the considerably higher 10 mg/m^3 concentration is in response to overload of normal or usual metabolic pathways and/or defense mechanisms. It is well recognized that a DNA adduct is not equivalent to a tumor (Swenberg et al., 1990). Adduction only informs the

investigator that something has happened within a cell. Without sophisticated techniques it is difficult to identify the adduct and to know its exact source. Adduct formation *per se* is not predictive of outcome. In the case of ETS, delivery of ADSS at concentrations over 100 fold that of a "typical" human exposure is not relevant.

6.2.4. Inhalation Studies of Cytochrome P450 and Clastogenesis

The measurement of lung cytochrome P450s 1A1 and 2B1 activities following exposure of rats to ADSS (Gebremichael et al., 1995) demonstrate that P4501A1 can be induced. The significance of this observation for humans exposed to ETS is unclear because of the high exposure to ADSS (i.e., 1.0 mg/m3).

The observation of clastogenesis by Mohtashamipur et al. (1987) has not been confirmed. The methodology used is inadequately documented. The exposure conditions and concentrations are unclear and the experimental design and methodology appear to be inappropriate. There is insufficient information to evaluate this paper.

6.2.5. Summary of Inhalation Studies with ADSS

A review of the most relevant studies in which animals were exposed via inhalation to ADSS, the most appropriate surrogate for ETS, reveals no association between ETS exposure and lung cancer. Any observed changes are consistent with reactive and adaptive responses. Abstracts of each of the studies follow in section 6.3.

6.3. Abstracts of Studies with ADSS

6.3.1. Clastogenic effect of passive smoking on bone marrow polychromatic erythrocytes of NMRI mice (Mohtashamipur et al., *Toxicol Lett*, 1987)

> The genotoxic effect of passive inhalation of sidestream cigarette smoke on bone marrow polychromatic erythrocytes was studied using male NMRI mice.

The animals were placed in individual 145.2-dm3 glass chambers resembling a room provided with normal air flow. They were exposed to the sidestream smoke of a commercial brand of cigarettes smoked by a smoking machine under standard conditions. Increased formation of micronuclei within polychromatic erythrocytes (PCEs) of femoral bone marrow 30 h after passive smoking was regarded as being due to the clastogenic effect of the smoke. Passive inhalation of the diluted sidestream smoke of a single cigarette resulted in a significant increase (P less than 0.01) in the frequency of micronucleated PCEs. This clastogenic activity was found to be dose-dependent.

6.3.2. Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study (von Meyerinck et al., *Exp Pathol,* 1989)

A 90-day feasibility study was performed in which rats and hamsters were exposed to the sidestream smoke of cigarettes. The only histopathological changes observed were hyperplasia and metaplasia of the epithelium covering the dorsal nasal turbinate bones in rats. These effects were reversible within 90 days. [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.3. Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. I. Inhalation toxicology and histopathology (Coggins, et al., *Fundam Appl Toxicol,* 1992)

Sprague-Dawley rats were exposed 6 hr per day for 14 consecutive days to aged and diluted sidestream smoke (ADSS), used as a surrogate for Environmental Tobacco Smoke (ETS), at concentrations of 0.1 (typical), 1 (extreme), or 10 (exaggerated) mg of particulates per cubic meter. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End-points included histopathology, CO-oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The only pathological response observed was slight to mild epithelial hyperplasia and inflammation in the most rostral part of the nasal cavity, in the high-exposure group only. No effects were noted at medium or low exposures. The minimal changes noted were reversible, using a subgroup of animals kept without further treatment for an additional 14 days. Overall, the end-points used in the study demonstrated that there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations and that the activity was only minimal at very exaggerated concentrations (particle concentrations 100 times higher than typical real-world concentrations). [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.4. Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. II. DNA adducts and alveolar macrophage cytogenetics (Lee et al., *Fundam Appl Toxicol,* 1992)

> The chemical constituents of cigarette smoke are greatly diluted in environmental tobacco smoke (ETS). In the typical indoor environment where cigarettes are smoked, the mean value of respirable suspended particles is approximately 0.1 mg/m3. In this study, we used aged and diluted sidestream smoke (ADSS) of 1R4F University of Kentucky research cigarettes as a surrogate for ETS and exposed Sprague-Dawley rats nose-only to 0, 0.1, 1.0, and 10 mg wet total particulate matter (WTPM)/m3 for 6 hr per day for 14 consecutive days. DNA from lung, heart, larynx, and liver was tested for adduct formation after 7 and 14 days of exposure and after 14 days of recovery. In addition, alveolar macrophages from animals exposed for 7 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any of the animals at 0.1 or 1.0 mg WTPM/m3, which represent ambient and 10-fold exaggerated ETS concentrations, respectively. Slight diagonal radioactive zones, characteristic of adducts observed in human smokers and in animals exposed to mainstream smoke, were observed, but only in lung and heart DNA of animals exposed to the highest concentration of ADSS (10 mg WTPM/m3), a 100-fold exaggeration of typical field measurements of ETS. The mean relative adduct labeling values (+/- SE) were 8.7 (+/- 0.2) adducts per 10(9) nucleotides for lung DNA and 5.7 (+/- 0.7) adducts per 10(9) nucleotides for heart DNA after 14 days of exposure. No elevation in chromosomal aberrations was observed in alveolar macrophages.(ABSTRACT TRUNCATED AT 250 WORDS) [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.5. Subchronic inhalation study in rats using aged and diluted sidestream smoke from a reference cigarette (Coggins et al., *Inhal Toxicol,* 1993)

> Male Sprague-Dawley rats were exposed 6 hr/day, 5 days/week for up to 13 weeks to aged and diluted sidestream smoke (ADSS), used as a surrogate for environmental tobacco smoke (ETS), at concentrations of 0.1 ("typical"), 1 ("extreme"), or 10 ("exaggerated") mg of particulates/m^3. Subgroups of animals were killed after 1 and 4 weeks of exposure. Animals were exposed nose-only, inside whole-body chambers, to ADSS from the 1R4F reference cigarette. End points included histopathology, CO oximetry, plasma nicotine and cotinine, clinical pathology, and organ and body weights. The target particulate concentrations were achieved; at the exaggerated exposure they resulted in CO concentrations in excess of 50 ppm. Particle size distributions showed that the aerosols were completely respirable: the mass median

diameter values were less than 1 μm. The only pathological response observed was slight to mild epithelial hyperplasia in the rostral nasal cavity, in the exaggerated exposure group only. No effects were noted at low (typical of measured real-world ETS concentrations) or extreme exposures. The changes were similar in animals killed after 4, 28, or 90 days, and were also similar to those noted in an earlier experiment with only 14 days duration, indicating that the change does not progress with increased exposure duration from 4 to 90 days. The nasal change was absent in a subgroup of animals kept without further smoke exposure for an additional 90 days, indicating complete reversibility. Overall, the end points used in the study demonstrated that (1) there was no detectable biological activity of ADSS at typical or even 10-fold ETS concentrations, and (2) the activity was only minimal at exaggerated concentrations in one region of one organ only. Based on the nasal histopathology, the NOEL for the 90-day study is >1 mg/m^3.

6.3.6. Ninety-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette: DNA adducts and alveolar macrophage cytogenetics (Lee et al., *Fundam Appl Toxicol.* 1993)

To study the genotoxic effects of subchronic exposure to environmental tobacco smoke, Sprague-Dawley rats were exposed to 0, 0.1, 1.0, and 10 mg total particulate matter (TPM)/m3 of aged and diluted sidestream smoke (ADSS) from 1R4F reference cigarettes 6 hr per day, 5 days a week for 13 weeks. DNA from lung, heart, larynx, bladder, and liver was tested for adduct formation by the 32P-postlabeling assay after 28 (except bladder) and 90 days of exposure and 90 days after cessation of exposure. In addition, alveolar macrophages from animals exposed for 28 or 90 days were examined for chromosomal aberrations. Exposure-related DNA adducts were not observed in any tissue in any of the animals exposed to 0.1 or 1.0 mg TPM/m3. However, increased levels of DNA adducts with diagonal radioactive zones were observed in lung, heart, and larynx DNA of animals exposed to the highest concentration of ADSS (10 mg TPM/m3). Adduct analyses with varying amounts of DNA from lungs of mid- and high-exposure animals clearly indicate that the dose-response for DNA adduct To study the genotoxic effects of subchronic exposure to environmental formation is nonlinear. The adduct levels were highest after 90 days of exposure and were significantly reduced in all target tissues 90 days after cessation of exposure. Chromosomal aberrations in alveolar macrophages were not elevated in any group after 28 or 90 days of exposure. These results indicate a no-observed-effect-level (NOEL) of at least 1.0 mg/m3 for DNA adduct formation in lung, heart, and larynx, and a NOEL of at least 10 mg/m3 for the induction of chromosome aberrations in alveolar macrophages, under the conditions of this study. [COPYRIGHT DIALOG(R)File 155:MEDLINE(R)]

6.3.7 Histopathological findings in the rat and hamster respiratory tract in a 90-day inhalation study using fresh sidestream smoke of the standard reference cigarette 2R1 (Teredesai and Pruhs, *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract,* ICSI Press, Washington, DC, 1994)

The reserve cell hyperplasia of the rat nasal respiratory epithelium and the lack of findings for the hamster are in accordance with published literature (von Meyerinck et al. 1989, Coggins et al. 1992, 1993). The slight hyperplasia and the slight squamous metaplasia found in the rat laryngeal epithelium have not been reported to date in the literature. The changes were reversible and are considered to be an adaptive response to repeated irritation. The No Observed Effect Level (NOEL) for all FSS-related findings for this study is between 2 and 6 μg TPM/L for rats. This concentration range is between 1 and 2 orders of magnitude above the average environmental concentration.

6.4.8. Short-term effects of sidestream smoke on respiratory epithelium in mice: cell kinetics (Rajini and Witschi, *Fundam Appl Toxicol,* 1994)

Male strain A/J and C57BL/6 mice were exposed on five consecutive days, for 6 hr a day, to sidestream smoke generated from Kentucky 1R4F reference cigarettes. Chamber concentrations were 1 mg/m3 of total suspended particulate matter and 528 to 549 micrograms/m3 of nicotine. Cumulative labeling indices in the airways and in the pulmonary parenchyma were measured following 1, 3, or 5 days exposure to Earlier studies have shown that both mainstream and sidestream cigarette smoke increase the activities of cytochrome P4501A1 and 2E1 in the lungs of adult animals; however, little information is available on the influence of ambient levels of sidestream cigarette smoke on cytochrome P450 monooxygenase activity in the developing lung. The present studies were conducted to define the developmental profiles of cytochrome P450 monooxygenases 1A1 and 2B1 in rat lung and liver and to assess the effects of aged and diluted sidestream cigarette smoke (ADSS) on the developmental profile of these two enzymes. Accordingly, pulmonary and hepatic microsomal P4501A1 and 2B1 activities were determined by measuring ethoxy- and pentoxyresorufin-O-delakylase (EROD and PROD, respectively) activity in animals exposed to filtered air or ADSS from birth to 7, 14, 21, 50, and 100 days of age. Pulmonary P4501A1 activity in control rats was not detected until 14 days of age. Activities increased threefold between 14 and 21 days of age and remained unchanged to 100 days of age. In animals exposed to ADSS from birth, pulmonary EROD activities were detected as early as 7 days postnatal and were elevated

three- to fourfold above control at all other ages examined. Hepatic EROD activities were unaltered by ADSS exposure. Short-term (4-day) ADSS exposure was as effective in upregulating pulmonary microsomal EROD activities as 100 unfiltered or filtered sidestream smoke. A significantly increased labeling index was found in A/J mice in the epithelium lining large intrapulmonary airways and terminal bronchioles after 3 and 5 days exposure to unfiltered smoke, whereas following exposure to filtered smoke labeling indices remained normal. The alveolar labeling index was not increased following smoke exposure. In C57BL/6 mice, sidestream smoke did not produce signs of increased cell proliferation in the respiratory tract. It is concluded that the response to sidestream smoke inhalation in mice may depend upon the strain of mice

6.3.9. Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data (Witschi, et al., *Fundam Appl Toxicol,* 1995)

Male strain A/J mice were exposed to sidestream smoke (SS) generated from burning Kentucky 1R4F reference cigarettes. Chamber concentrations were 4 mg/m3 of total suspended respirable particulate matter (TSP). Animals were exposed 6 hr a day, 5 days a week. One-week cumulative labeling indices were significantly increased in the large intrapulmonary airways during the 1st week and in the respiratory epithelium of the nasal and maxillar turbinates during the first 3 weeks of exposure and then returned to control values. Subsequently, signs of increased cell proliferation were again found in the nasal and maxillar turbinates during the 9th and 16th exposure weeks. The experiment was terminated after 6 months. The number of animals bearing lung tumors was the same in smoke-exposed as in filtered air-exposed animals as was the average number of tumors per lung. Analysis of the DNA of individual tumors obtained from exposed and control mice for K-ras mutations suggested that exon 2 might be a specific target for SS. It was concluded that (1) duration of exposure was too short or (2) concentration of TSP was too low to reveal a possible carcinogenic potential of SS in strain A/J mice or that (3) SS is not carcinogenic in strain A mice.

6.3.9. Replicative DNA synthesis in tissues of the rat exposed to aged and diluted sidestream smoke (Ayres et al., *Inhalation Toxicology,* 1995)

Male Sprague-Dawley rats were exposed to aged and diluted sidestream smoke (ADSS) from Kentucky 1R4F reference cigarettes for 6 h/day, 5 days/wk, for a 13-wk period. Exposure concentrations were 0, 0.1, 1, and 10 mg ADSS/m^3. Exposures were conducted in whole-body inhalation chambers. Rats were held in nose-only exposure tubes for the 6-h exposures to minimize pelt deposition and subsequent ingestion of ADSS. Groups of 10

rats from each exposure group were killed after 5, 28, and 90 d of exposure to examine the rates of replicative DNA synthesis; 6 rats from each exposure group were kept for a 90-day recovery period after termination of exposures to examine replicative DNA synthesis rates. Three days prior to each scheduled necropsy, an osmotic pump containing 5-bromo-2'-deoxyuridine (BrdU) was implanted subcutaneously. After necropsy, tissues were processed for examination of BrdU-containing cells at several sites. Incorporation of BrdU was assessed either by counting the number of labeled cells along a length of an epithelial surface or by counting the number of labeled cells in an area of tissue. Tissues examined were from the nasal cavity, ventral larynx, and trachea, in addition to bronchial, bronchiolar, and alveolar regions of the lung. Endocardium, myocardium, epicardium, and aortic smooth muscle sites were also examined. Increased replicative DNA synthesis occurred in some sites of the respiratory tract at the 5-day time point at the mid or high exposure concentrations, although at 28 and 90 days, these effects had diminished in intensity or were not present, indicating adaptation to the ADSS exposure. The only tissues with elevated rates of replicative DNA synthesis at 90 days were the cuboidal and respiratory epithelium at the most rostral portion of the nasal cavity at the highest exposure concentration. Increased rates of replicative DNA synthesis were not noted in heart tissues or lung alveolar epithelium at any concentration at any time point. Examination of rats killed after the end of the 90-day recover period indicated that the increase in replicative DNA synthesis was not sustained after termination of exposures. The no observed effect level (NOEL) for increased replicative DNA synthesis after subchronic exposure to ADSS in the rate is greater than 1 mg ADSS/m^3

6.3.11. Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: effect of aged and diluted sidestream cigarette smoke (Gebremichael, et al., *Toxicol Appl Pharmacol,* 1995)

Earlier studies have shown that both mainstream and sidestream cigarette smoke increase the activities of cytochrome P4501A1 and 2E1 in the lungs of adult animals; however, little information is available on the influence of ambient levels of sidestream cigarette smoke on cytochrome P450 monooxygenase activity in the developing lung. The present studies were conducted to define the developmental profiles of cytochrome P450 monooxygenases 1A1 and 2B1 in rat lung and liver and to assess the effects of aged and diluted sidestream cigarette smoke (ADSS) on the developmental profile of these two enzymes. Accordingly, pulmonary and hepatic microsomal P4501A1 and 2B1 activities were determined by measuring ethoxy- and pentoxyresorufin-O-delakylase (EROD and PROD, respectively) activity in animals exposed to filtered air or ADSS from birth to 7, 14, 21, 50,

and 100 days of age. Pulmonary P4501A1 activity in control rats was not detected until 14 days of age. Activities increased threefold between 14 and 21 days of age under manned unchanged to 100 days of age. In animals exposed to ADSS from birth, pulmonary EROD activities were detected as early as 7 days postnatal and were elevated three- to fourfold above control at all other ages examined. Hepatic EROD activities were unaltered by ADSS exposure. Short-term (4-day) ADSS exposure was as effective in upregulating pulmonary microsomal EROD activities as 100-day exposures. Induction of pulmonary EROD activities and the associated increases in mRNA levels were dependent upon the particulate fraction. Stimulation of EROD activities in major and minor daughter subcompartments was there- to fourfold higher in ADSS-exposed animals compared to controls, while there was no induction in the trachea and less than a twofold increase in the parenchyma. Pulmonary PROD activities developed more slowly than EROD and did not reach adult levels until Day 50. ADSS did not alter pulmonary or hepatic PROD activities. These studies show that P4501A1 and 2B1 develop at different rates in rat lung and liver and that exposure to ADSS markedly increases P4501A1 activities in the lung at all ages examined.

7. REFERENCES

Ames BN, Shigenaga MK, Gold LS (1993). DNA lesions, inducible DNA repair, and cell division: Three key factors in mutagenesis and carcinogenesis. *Environmental Health Perspectives* **101**: 35-44

Aviado DM (1995). Occupational lung disease and environmental tobacco smoke. *Internaional Journal of Occupational Medicine and Toxicology* **4**: 383-407

Ayres PH, McKarns SC, Coggins CRE, Doolittle DJ, Sagartz JE, Payne VM, Mosberg AT (1995). Replicative DNA synthesis in tissues of the rat exposed to aged and diluted sidestream smoke. *Inhalation Toxicology* 7: 1225-1246

Barrett JC (1993). Mechanisms of multistep carcinogenesis and carcinogen risk assessment. *Environmental Health Perspectives* **100**: 9-20

Beck BD, Rudel R, Calabrese EJ (1994). The use of toxicology in the regulatory process. In: Hayes AW, ed. Principles and methods of toxicology. New York: Raven Press, Ltd, pp 28-40

Burger GT, Renne RA, Sagartz JW, Ayres PH, Coggins CRE, Mosberg AT, Hayes AW (1989). Histologic changes in the respiratory tract induced by inhalation of xenobiotics: physiologic adaptation or toxicity? *Toxicology and Applied Pharmachology* **101**: 521-542

Butterworth BE, Conolly RB, Morgan KT (1995). A strategy for establishing mode of action of chemical carcinogens as a guide for approaches to risk assessments. *Cancer Letters* **93**: 129-146

Calabrese EJ, ed. (1992). Bilogical effects of low level exposures to chemicals and radiation. Boca Raton: Lewis Publishers

Coggins CRE, Ayres PH, Mosberg AT, Ogden MW Sagartz JW, Hayes AW (1992). Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. I. Inhalation toxicology and histopathology. *Fundamental and Applied Toxicology* **19**: 133-140

Coggins CRE, Ayers PH, Mosberg AT, Sagartz JW, Hayes AW (1993). Subchronic inhalation study in rats using aged and diluted sidestream smoke from a reference cigarette. *Inhalation Toxicology* **5**: 77-96

Cohen BL (1995). How dangerous is low level radiation? *Risk Analysis* **15**: 645-653

Dalbey WE, Nettesheim P,Griesemer R, Caton JE, Guerin MR (1980). Chronic inhalation of cigarette smoke by F344 rats. *Journal National Cancer Institute* **64**: 383-390

Faccini JM, Butler WR, Friedmann JC, Hess R, Reznik, Ito N, Hayashi Y, Williams GM (1992). IFSTP guidelines for the design and interpretation of the chronic rodent carcinogenicity bioassay. *Experimental Toxicology and Pathology* **44**: 443-456

Farber E (1992). Cellular adaptation as an important response during chemical carcinogenesis. In: Calareses EJ, ed. Bilogical effects of low level exposures to chemicals and radiation. Boca Raton: Lewis Publishers, pp 113-118

Gebremichael A, Chang AM, Buckpitt AR, Plopper CG, Pinkerton KE (1995). Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: effect of aged and diluted sidestream cigarette smoke. *Toxicology and Applied Pharmacology* **135**: 246-253

Gori GB, Mantel N (1991). Mainstream and environmental tobacco smoke. *Regulatory Toxicology and Pharmacology* **14**: 88-105

Guerin MR, Higgins CE, Griest WH (1987). The analysis of the particulate and vapour phases of tobacco smoke. In: O'Neill IK, Burnnemann KD, Dodet B, Hoffman D, eds. Environmental carcinogens methods of analysis and exposure measurement. Volume 9 Lyon: International Agency for Research on Cancer, pp 115-139

Guerin MR, Jenkins RA, Tomkins BA (1992). The chemistry of environmental tobacco smoke: composition and measurement. Boca Raton: Lewis Publishers

Guerin MR (1987). Formation and physicochemical nature of sidestream smoke. In: O'Neill IK, Brunnemann KD, Dodet B, Hoffmann D, eds. Environmental carcinogens methods of analysis and exposure measurement. Lyon: International Agency for Research on Cancer, pp 11-23

Hattermer-Frey HA, Travis CC (1991). Benzo-a-pyrene: environmental partitioning and human exposure. *Toxoicology and Industrial Health* 7: 141-157

Heckman CA, Dalbey E (1982). Pathogenesis of lesions induced in reat lung by chronic tobacco smoke inhalation. *Journal of National Cancer Organization* **1**: 117-129

Heckman CA, Lehman GL (1985). Ultrastructure and distribution of intracellular spicules in rat lung following chronic tobacco smoke exposure. *Journal National Cancer Institute* **74**: 647-657

Henry CJ, Kouri RE (1986). Chronic inhalation studies in mice. II. Effects of long-term exposure to 2R1 cigarette smoke on (C57BL/Cum X C3H/AnfCum)F1 mice. *Journal National Cancer Institute* 77: 203-212

Hiller FC (1984). Deposition of sidestream cigarette smoke in the human respiratory tract. *Preventive Medicine* **13**: 602-607

Holcomb LC (1993). Indoor air quality and environmental tobacco smoke: concentration and exposure. *Environmental International* **19**: 9-40

Hoffman D, Hecht SS (1990). Advances in tobacco carcinogenesis. In: Cooper CS, Grover PL, eds. Chemical carcinogenesis and mutagenesis I. New York: Springer-Verlag, pp 85-102

Hrudey SE, Krewski D (1995). Is there a safe level of exposure to a carcinogen? *Enviornmental Science & Technology* **29**: 370-375

Huber GL (1989). Physical, chemical, and biologic properties of tobacco, cigarette smoke, and other tobacco products. *Seminars in Respiratory Medicine* **10**: 297-332

International Agency for Research on Cancer (IARC, 1983). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Volume 32. Lyon, France: World Health Organization

International Agency for Research on Cancer (IARC, 1986). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans: tobacco smoking, Volume 38. Lyon, France: World Health Organization.

International Agency for Research on Cancer (IARC, 1992). Mechanisms of carcinogenesis in risk identification. IARC Scientific Publications No. 116. Lyon: World Health Organization

Lee CK, Brown BG, Reed BA, Rahn CA, Coggins CRE, Doolittle DJ, Hayes AW (1992). Fourteen-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette. *Fundamental and Applied Toxicology* **19**: 141-146

Lee CK, Brown BG, Reed EA, Coggins CRE, Doolittle DJ, Hayes AW (1993). Ninety-day inhalation study in rats, using aged and diluted sidestream smoke from a reference cigarette: DNA adducts and alveolar macrophage cytogenetics. *Fundamental and Applied Toxicology* **20**: 393-401

Lee PN, Forey BA (1995). Misclassification of smoking habits as determined by continine or by repeated self-report - A summary of evidence from 42 studies. *Journal of Smoking-Related Diseases* **6**: 109-129

Lofroth G, Burton RM, Forehand L, Hammond SK, Sella RL, Zweidinger RB, Lewtas (1989). Characterization of Environmental Tobacco Smoke. *Environ Sci Technol* **23**: 610-614

McAughey JJ, Knight DA, Black A, Dickens CJ (1994). Environmental tobacco smoke retention in humans from measurements of exhaled smoke composition. *Inhalation Toxicology* **6**: 615-631

von Meyerinck L, Scherer G, Adlkofer F, Wenzel-Hartung R, Brune H, Thomas C (1989). Exposure of rats and hamsters to sidestream smoke from cigarettes in a subchronic inhalation study. *Experimental Pathology* **37**: 1-4

Mohtashamipur E, Norpoth K, Straeter H (1987). Clastogenic effect of passive smoking on bone marrow polychromatic erythocytes of NMRI mice. *Toxicology Letters* **35**: 153-156

National Research Council (NRC, 1986). Environmental tobacco smoke: measuring exposures and assessing health effects. Washington, DC: National Academy Press.

Phalen RF (1984a). Aerosols and gases. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 1-31

Phalen RF (1984b). Testing for toxicity. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 151-172

Phalen RF (1984c). Animal models. In: Inhalation studies: Foundations and techniques. Boca Raton: CRC Press, Inc., pp 211-241

Pitot III HC, Dragan YP (1996). Chemical Carcinogenesis. In: Klaassen CD, Amdur MA, Doull J, eds. Casarett and Doull's toxicology: the basic science of poisons. New York: McGraw-Hill, pp 201-267

Purchase IFH, Auton TR (1995). Thresholds in chemical carcinogenesis. *Regulatory Toxicology and Pharmachology* **22**: 199-205

R.J. Reynolds Tobacco Company (RJR, 1986). Chemical and bilogical studies on new cigarette prototypes that heat instead of burn tobacco. Winston Salem: R. J. Reynolds Tobacco Company, pp 151-170

Rajini P, Witschi H (1994). Short-term effects of sidestream smoke on respiratory ephithelum in mice: cell kinetics. *Fundamental and Applied Toxicology* **22**: 405-410

Rodgman A (1992). Environmental tobacco smoke. *Regulatory Toxicology and Pharmacology* **16**: 223-244

Roe FJC, Perry R (1991). The physical and chemical characteristics of environmental tobacco smoke with special reference to exposure dose. In: Armitage AK, ed. Other people's tobacco smoke. E. Yorks: Galen Press, pp 21-39

Sagan L (1994). The triumph of theology over science: the non-threshold effects model. *International Journal of Occupational Medicine and Toxicology* **3**: 161-172

Scherer G, Conze C, v Meyerinck L, Sorsa M, Adlkofer F (1990). Importance of exposure to gaseous and particulate phase components of toacco smoke in active and passive smokers. *International Archives of Occupational and Environmental Health* **62**: 459-466

Spilker B (1991). Concept of cause and effect. In: Guide to clinical trials. New York: Raven Press, Ltd., pp 528-535

Sugita M, Izuno T, Kanamori M (1995). Recalculation of summarised odds ratios for the relationship between passive smoking and lung cancer based on data in the EPA report. *Indoor Environ* **4**: 177-181

Swenberg JA, Fedtke N, Fennell TR, Walker VE (1990). Relationships between carcinogen exposure, DNA adducts and carcinogenesis. In: Clayson DB, Munro IC, Shubik P, Swenerg JA, eds. Progress in predictive toxicology. New York: Elsevier Science Publishers B.V., pp 161-184

Teredesai A, Pruhs D (1994). Histopathological findings in the rat and hamster respiratory tract in a 90-day inhalation study using fresh sidestream smoke of the standard reference cigarette 2R1 In: Dungworth DL, Mauderly JL, Oberdorser G, eds. Toxic and carcinogenic effects of solid particles in the respiratory tract. Washington, D.C.: International Life Sciences Institute, pp 629-635

Trosko JE, Chang CC (1988). Nongenotoxic mechanisms in carcinogenesis: role of inhibited intercellular communication. Bradbury Report 31 Carcinogen risk assessment: new directions in the qualitative and quantitative aspects. New York: Cold Spring Harbor Laboratory, pp 139-170

U.S. Department of Health and Human Services (U.S. DHHS, 1986). Report of the Surgeon General: The Health Consequences of Involuntary Smoking. DHHS Pub No (PHS) 87-8398. Washington, D.C.

U.S. Environmental Protection Agency (U.S. EPA, 1992). Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders. Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.

Upton AC (1988). Are there thresholds for carcinogenesis? The thorny problem of low-level exposure. *Annals of the New York Academy of Sciences* **534**: 563-884

Valberg PA, Blanchard JD (1991). Pulmonary macrophage physiology: origin, motility, endocytosis. In: Parent RA, ed. Comprehensive treatise on pulmonary toxicology. Boca Raton: CRC Press, pp 681-724

Waldman JM, Lioy PJ, Greenberg A, Butler JP (1991). Analysis of human exposure to benzo(a)pyrene via inhalation and food ingestion in the total human environmental exposure study (THEES). *Journal of Exposure Analysis and Environmental Epidemiology* **1**: 193-225

Witschi H, Oreffo VI, Pinkerton KE (1995). Six-month exposure of strain A/J mice to cigarette sidestream smoke: cell kinetics and lung tumor data. *Fundamental and Applied Toxicology* **26**: 32-40

Witshi H, Pajini P (1994). Cell inetics in the respiratory tract of hamsters exposed to sidestream smoke. *Inhalation Toxicology* **6**: 321-333

Wydner EL, Hoffman D (1967). Toabcco and tobacco smoke. New York: Academic Press

Wynder EL, Williams GM (1992). Metabolic overload and carcinogenesis from the viewpoint of epidemiology. *bga-Schriften* **3**: 17-24

Zeise L, Wilson R, Crouch EAC (1987). Dose-response relationships for carcinogens: a review. *Environmental Health Perspectives* **73**: 259-308

APPENDIX I

CURRICULUM VITAE OF SUSAN A. RICE, PH.D.

SUSAN A. RICE AND ASSOCIATES, INC.

Consultants in Toxicology and Pharmacology

CURRICULUM VITAE

SUSAN A. RICE, PH.D., D.A.B.T.

EDUCATION AND PROFESSIONAL CERTIFICATION

Diplomate of the American Board of Toxicology (1990-Present)
Ph.D. in Comparative Pharmacology and Toxicology, University of California, Davis (1976)
B.S. in Biochemistry, University of California, Davis (1971)

SUMMARY

Professional competence encompasses general toxicology and pharmacology including clinical, environmental, occupational, and product toxicology.

Areas of practice include product safety evaluation, exposure and health assessments for various routes of exposure, litigation support, and scientific support and audit for regulatory submission. Specialty areas include anesthetic toxicity, hepatotoxicity, nephrotoxicity, neurotoxicity, reproductive toxicity, developmental toxicity, teratology (morphologic and behavioral), biotransformation of drugs and chemicals, and molecular mechanisms of toxicologic and pharmacologic action.

Agents and devices evaluated include chemicals, air pollutants (indoor and outdoor), pharmaceuticals, biologics, biotechnology products, pesticides, physical agents (radiation, heat, etc.), and medical devices.

Services include identification of hazardous chemicals and/or agents, assessment of exposure, evaluation of dose-response relationships, characterization of potential risk, identification of conditions adversely affecting health, design of health studies, and analysis and interpretation of data and scientific literature. Evaluations are both quantitative and qualitative in nature depending on the quality and quantity of available scientific data.

CONSULTING ACTIVITIES

The following are selected examples of work to evaluate and interpret:

- Airborne methylacrylate, isocyanate, and chlorine exposures and potential health effects
- Medical records and eyewitness accounts to determine cause of injury or death (traumatic, asphyxia, etc.) and related signs and symptoms of alleged toxic exposure
- Health effects of indoor exposures to carbon monoxide, chemicals, and microbial pathogens
- Potential exposures to toxic waste materials
- Alleged pesticide exposures
- Injuries from dermal and inhalational exposure to multiple solvents

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Sunnyvale, CA 94087-0299
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CONSULTING ACTIVITIES (continued)

- Effects of carbon monoxide on health, neurological, and cognitive function
- Sewer gases and potential health risks
- Alleged exposure and adverse health effects of combustion products
- Lead exposures, blood lead concentrations, and neurological and cognitive outcome
- Potential interactions of calcium channel-blocking and beta-blocking drugs
- Bacterial contamination of intravenous drugs
- Health effects of medical devices including IUDs and breast implants
- Risk factors leading to diseases of various organs and organ systems

POSITIONS AND APPOINTMENTS

Susan A. Rice and Associates, Inc., Sunnyvale, CA
Toxicology and Pharmacology
President (1993-Present)

Failure Analysis Associates, Inc., Menlo Park, CA
Toxicology and Pharmacology
Senior/Managing Scientist (1990-1993)

SRI International, Menlo Park, CA
Biochemical Toxicology and Pharmacology
Senior Toxicologist, for conduct of NIH funded research (1990-1992)

Stanford University School of Medicine, Stanford, CA
Department of Anesthesia
Consulting Associate Professor of Pharmacology and Toxicology in Anesthesia (1990-Present)
Assistant/Associate Professor of Pharmacology and Toxicology in Anesthesia (1979-1990)
Postdoctoral Fellow/Research Associate in Anesthesia, Pharmacology and Toxicology (1976-1979)

Veterans Administration Medical Center (PAVAMC), Palo Alto, CA
Anesthesiology and Research Services
Associate Research Career Toxicologist/Pharmacologist (1987-1990)
Research Pharmacologist (1979-1987)
Postdoctoral Fellow/Research Associate in Anesthesia, Pharmacology and Toxicology (1976-1979)

School of Veterinary Medicine, University of California, Davis, CA
Department of Physiological Sciences
Walter Foster Fellow for Pulmonary Research (1974-1976)
Research Assistant/Laboratory Helper and Assistant I and II and Independent Study (1968-1974)

School of Public Health, University of California, Berkeley, CA
Department of Environmental Health Sciences
Assistant Specialist, Step II (1975-1976)

School of Medicine, University of California, Davis, CA
Department of Internal Medicine
Postgraduate Research Physiologist I (1971-1972)
California TB and Respiratory Disease Association Research Fellowship (1971)

PROFESSIONAL SOCIETY MEMBERSHIPS AND OFFICES

American Society of Anesthesiologists
American Society for Pharmacology and Experimental Therapeutics
California Society of Anesthesiologists
Genetic and Environmental Toxicology Association of Northern California
International Society for the Study of Xenobiotics
Neurobehavioral Teratology Society [formerly, Behavioral Teratology Society]
- President (1993-1994); President Elect (1992-1993)
- Secretary (1988-1992)
- Publications Committee (1990-1992; 1996-1999)
- Nominating Committee (1984-1985)

Northern California Chapter, Society of Toxicology
- Membership Committee (1991-1994), Chairperson (1991-1993)
- Nominating Committee (1986-1989)
- Treasurer (1995-1997)

Society for Neuroscience
Society of Toxicology
Teratology Society
Western Pharmacology Society
Western Teratology Society

VOLUNTARY SERVICE

California Environmental Protection Agency
- Comparative Risk Project for California, Human Health Subcommittee, Toxicology Dose-Response Work Group (1992-1994)

Stanford University School of Medicine
- Faculty Senate (1981-1989)
- Admissions Committee/Minority Admissions Committee (1979-1983)

Stanford University School of Medicine, Department of Anesthesia
- Committee on Resident Education (1980-1990)
- Committee on Medical Student Education (1983-1988)
  - Acting Chairperson (1984-1985)
- Committee on Biosafety (1979-1983)

Veterans Administration Medical Center
- Subcommittee on Safety for Medical Research Laboratories (1979-1990)
  - Chairperson (1989-1990)
- Occupational Health Consultant (1983-1990)
- Research and Development Subcommittee on Animal Studies (1983-1989)
  - Chairperson (1986-1989)

RESEARCH FUNDING

National Institute of General Medical Sciences # RO-1-22746
- PI - Nephrotoxicity of Fluorinated Anesthetics (1984-1985; 1985-1988; 1988-1992)
- Co-PI (Co-PI, R. Mazze) - Nephrotoxicity of Fluorinated Anesthetics (1982-1984)
- Investigator (PI, R. Mazze) - Nephrotoxicity of Fluorinated Anesthetics (1979-1982)
- Investigator (PI, R. Mazze) - Nephrotoxicity of Fluorinated Volatile Anesthetics (1977-1979)

RESEARCH FUNDING (continued)

National Institutes of Health, Biomedical Research Support Grant, Stanford University # 5353
PI - Characterization of Isoniazid-Induced Defluorinase Activity (1981-1982)

Stanford University School of Medicine, Department of Anesthesia Research Committee
PI - Behavioral Teratogenicity of Nitrous Oxide (1981)

Veterans Administration Merit Review Program
PI - Parental N_2O and Behavioral Effects and Mechanisms in Offspring (1987-1990)
PI - Behavioral Teratogenicity of Inhaled Anesthetic Agents (1981-1984; 1984-1987)
Investigator (PI, R. Mazze) - Anesthetic Toxicity and Metabolism (1977-1979; 1979-1983; 1983-1985)

University of California, Davis, Chancellor's Patent Fund Grant
PI - Pulmonary Toxicity of Thiocarbamides (1973-1974; 1974-1975; 1975-1976)

REVIEWER

Anesthesiology
Neurotoxicology and Teratology
(Editorial Board, 1990-1992; 1995-1998)
Fundamental and Applied Toxicology
Teratology
Toxicology and Applied Pharmacology

HONORS AND AWARDS

Diplomate of the American Board of Toxicology (1990-present)
VA Associate Research Career Scientist Award (Salary) resigned in 1990 (1987-1992)
Walter Foster Fellowship for Pulmonary Research (1974-1975; 1975-1976)
Chancellor's Patent Fund Grant, UC, Davis (1973-1974; 1974-1975; 1975-1976)
Summer, California Tuberculosis and Respiratory Disease Fellowship, UC, Davis (1971)

PUBLICATIONS

1. Giri SN and Rice SA: Influence of phenoxybenzamine on gastric evacuation in rats. Life Sciences 9:1109-1115, 1970.

2. Cronin [Rice] SR, Giri SN and Zimmerman RA: Aberrations in carbohydrate metabolism in response to pulmonary edematogenics. Proc West Pharmacol Soc 17:256-261, 1974.

3. Cronin [Rice] SR and Giri SN: Effects of pulmonary irritants on DNA, ATPase activity and histamine in rat lungs. Proc Soc Exp Biol Med 146:120-125, 1974.

4. Devereux DF, Rice SA and Giri SN: The effect of thermal burns on blood and plasma histamine levels in the rat and the cat. Proc West Pharmacol Soc 18:114-118, 1975.

5. Rice SA and Giri SN: Effect of thiourea on pulmonary vascular leakage in rats of different ages. Proc West Pharmacol Soc 18:128-132, 1975.

6. Giri SN, Benson J, Siegel DM, Rice SA and Schiedt M: Effects of pretreatment with anti-inflammatory drugs on ozone-induced lung damage in rats. Proc Soc Exp Biol Med 150:810-814, 1975.

7. Giri SN, Rice SA and Bacchetti P: Characteristic features of actinomycin D-induced paw inflammation of the rat. J Mol Path 23:367-378, 1975.

8. Devereux DF, Michas CA and Rice SA: Heparin pretreatment suppresses norepinephrine concentrations in dogs in endotoxic shock. Clin Chem 23:1346-1347, 1977.

9. Devereux DF, Rice SA and Giri SN: The effects of heparin pretreatment on plasma histamine following thermal injury in rats and cats. Circ Shock 5:311-316, 1978.

10. Baden JM, Kelley M, Simmon VF, Rice SA and Mazze RI: Fluroxene mutagenicity. Mutat Res 58:183-191, 1978.

11. Shu H, Talcott RE, Rice SA and Wei ET: Lipid peroxidation and paraquat toxicity. Biochem Pharmacol 28:327-331, 1979.

12. Dooley JR, Mazze RI, Rice SA and Borel JD: Is enflurane defluorination inducible in man? Anesthesiology 50:213-217, 1979.

13. Baden JM, Mazze RI, Wharton RS, Rice SA and Kosek JC: Carcinogenicity of halothane in Swiss/ICR mice. Anesthesiology 51:20-26, 1979.

14. Caughey GH, Rice SA, Kosek JC and Mazze RI: Effect of phenytoin (DPH) treatment on methoxyflurane metabolism in rats. J Pharmacol Exp Ther 210:180-185, 1979.

15. Wharton RS, Wilson AI, Mazze RI, Baden JM and Rice SA: Fetal morphology in mice exposed to halothane. Anesthesiology 51:532-537, 1979.

16. Rice SA and Talcott RE: Effects of isoniazid treatment on selected hepatic mixed function oxidases. Drug Metab Disp 7:260-262, 1979.

17. Sievenpiper TS, Rice SA, McClendon FD, Kosek JC and Mazze RI: Renal effects of enflurane anesthesia in Fischer 344 rats with pre-existing renal insufficiency. J Pharmacol Exp Ther 211:36-41, 1979.

18. Baden JM, Egbert B and Rice SA: Enflurane has no effect on haemopoiesis in mice. Br J Anaesth 52:471-474, 1980.

19. Baden JM, Rice SA, Wharton RS and Laughlin NK: Metabolic and toxicologic studies with enflurane in Swiss/ICR mice. J Environ Pathol Toxicol 4:293-303, 1980.

20. Fish K, Sievenpiper T, Rice SA, Wharton RS and Mazze RI: Renal function in Fischer 344 rats with chronic renal impairment after administration of enflurane and gentamicin. Anesthesiology 53:481-488, 1980.

21. Rice SA, Sbordone L and Mazze RI: Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following isoniazid administration. Anesthesiology 53:489-493, 1980.

22. Rice SA and Fish MP: Effects of Isoniazid Metabolites On the Rate of Hepatic Microsomal Defluorination of Volatile Fluorinated Ether Anesthetics. In: Microsomes, Drug Oxidations and Chemical Carcinogenesis. Vol II, 885-888. Coon MJ, et al. (Eds). Academic Press, 1980.

23. Baden JM, Rice SA and Mazze RI: Deuterated methoxyflurane anesthesia and renal function in Fischer 344 rats. Anesthesiology 56:203-206, 1982.

24. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in mice chronically exposed to nitrous oxide. Teratology 26:11-16, 1982.

25. Rice SA, Dooley JR and Mazze RI: Metabolism by rat hepatic microsomes of fluorinated ether anesthetics following ethanol consumption. Anesthesiology 58:237-241, 1983.

26. Mazze RI, Rice SA, Wyrobek AJ, Felton JA, Brodsky JB and Baden JM: Germ cell studies in mice after prolonged exposure to nitrous oxide. Toxicol Appl Pharmacol 67:370-375, 1983.

27. Baden JM, Rice SA, Serra M, Kelley M and Mazze RI: Thymidine and methionine syntheses in pregnant rats exposed to nitrous oxide. Anesth Analg 62:738-741, 1983.

28. Fish KJ and Rice SA: Halothane inhibits metabolism of enflurane in Fischer 344 rats. Anesthesiology 59:417-420, 1983.

29. Rice SA, Sievenpiper TS and Mazze RI: Liver function and anesthetic metabolism in rats with chronic renal impairment. Anesthesiology 60:418-421, 1984.

30. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in rats exposed to nitrous oxide. Teratology 30:259-266, 1984.

31. Mazze RI, Rice SA and Baden JM: Halothane, isoflurane and enflurane MAC in mice and rats. Anesthesiology 62:339-341, 1985.

32. Rice SA, Mazze RI and Baden JM: Effects of subchronic intermittent exposure to nitrous oxide in Swiss Webster mice. J Environ Pathol Toxicol Oncology 6:271-281, 1985.

33. Mazze RI, Wilson AI and Rice SA: Fetal development in mice exposed to isoflurane. Teratology 32:339-345, 1985.

34. Rice SA, Baden JM and Kundomal YR: Effects of subchronic intermittent exposure to isoflurane in Swiss Webster mice. J Environ Pathol Toxicol Oncology 6:285-293, 1986.

35. Servin FF, Nivoche Y, Desmonts J, Rice SA and Mazze RI: Biotransformation of halothane and enflurane in patient with hyperthyroidism. Anesthesiology 64:387-391, 1986.

36. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden JM: Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. Anesthesiology 64:339-344, 1986.

37. Rice SA and Millan DP: Validation of a developmental swimming test using Swiss Webster mice treated perinatally with methimazole. Neurobehav Toxicol Teratol 8:69-75, 1986.

38. Rice SA and Fish KJ: Anesthetic metabolism and renal function in obese and non-obese Fischer 344 rats following enflurane or isoflurane anesthesia. Anesthesiology 65:28-34, 1986.

39. Wang S-L, Rice SA, Serra MT and Gross B: Purification and identification of rat cytosolic enzymes responsible for defluorination of methoxyflurane and fluoroacetate. Drug Metab Dispos 14:392-398, 1986.

40. Biermann JS, Rice SA, Gallagher E and West JA: Effect of diazepam treatment on hepatic microsomal anesthetic defluorinase activity. Arch Int Pharmacodyn Ther 283:181-192, 1986.

41. Rice SA, Maze M, Smith CM, Kosek JC and Mazze RI: Halothane hepatotoxicity in Fischer 344 rats pretreated with isoniazid. Toxicol Appl Pharmacol 87:411-419, 1987.

42. Rice SA, Millan DP and West JA: The behavioral effects of perinatal methimazole administration in Swiss Webster mice. Fund Appl Toxicol 8:531-540, 1987.

43. Fish KJ, Rice SA and Margary J: Contrasting effects of etomidate and propylene glycol upon enflurane metabolism and adrenal steroidogenesis in Fischer 344 rats. Anesthesiology 68:189-193, 1988.

44. Rice SA, Fish KJ, Hoover-Plow J, Jawaharlal K: In vitro hepatic drug and anesthetic metabolism of rats with dietary-induced obesity. Arch Int Pharmcodyn Ther, 299:286-293, 1989.

45. Biermann JS, Rice SA, Fish KJ and Serra MT: Metabolism of halothane in obese Fischer 344 rats. Anesthesiology 71:431-437, 1989.

46. Truog RD and Rice SA: Inorganic fluoride and prolonged isoflurane administration in he intensive care unit. Anesth Analg 69:843-845, 1989.

47. Pearl RG and Rice SA: Propylene glycol-induced pulmonary hypertension in sheep. Pharmacology 39:383-389, 1989.

48. Rice SA: Effect of prenatal N_2O exposure on startle reflex reactivity. Teratology 42:373-381, 1990.

49. Giri SN, Hollinger MA and Rice SA: Effects of thiourea on pulmonary vascular permeability and on lung and plasma histamine levels in rats. Toxicology Letters 57:283-290, 1991.

50. Giri SN, Hollinger MA and Rice SA: Effects of thiourea tolerance on plasma histamine, and lung vascular permeability. Archives of Toxicology 65:603-605, 1991.

51 Pearl RG, Baer ER, Siegel LC, Benson GV and Rice SA: Longitudinal distribution of pulmonary vascular resistance after endotoxin administration in sheep. Critical Care Medicine 20:119-125, 1992.

52. Hoover-Plow J and Rice S: Physical and behavioral development in two strains of mice fed excessive dietary vitamin B6. Nutrition Research 12:773-786, 1992.

53. Arnold JH, Troug RD and Rice SA: Prolonged administration of isoflurane to pediatric patients during mechanical ventilation. Anesth Analg 76:520-526, 1993.

ABSTRACTS

1. Mustafa MG, Cronin (Rice) SR, Duvall TR and Cross CE: Ozone effect on activities of lung cellular membrane enzymes. Clin Res 20:197, 1972.

2. Giri SN, Kartt LR, Joshi B and Cronin (Rice) SR: Mobilization of leucocytes and subsequent release of histamine and lysosomal enzymes into the peritoneal cavity and pleural effusions induced by the IP injection of actinomycin D. Fed Proc 33:558, 1974.

3. Giri SN, Kartt LR, Cronin (Rice) SR and Peoples SA: Inflammatory changes following subplantar administration of actinomycin D. Pharmacologist 16:292, 1974.

4. Rice SA and Giri SN: Histamine and pulmonary vascular permeability in tolerance to the lethal effects of thiourea. Pharmacologist 17:193, 1975.

5. Caughey G, Rice S, Hitt B and Mazze RI: Diphenylhydantoin (DPH) induction of methoxyflurane (MOF) metabolism in rats. Fed Proc 36:998, 1977.

6. Rice SA, Dooley JR and Mazze RI: Ethanol induction of hepatic microsomal defluorinase activity in rats. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, New Orleans, October 1977, pp. 349-350.

7. Baden JM, Kelley M, Mazze RI, Rice SA and Simmon VF: Fluroxene mutagenicity. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, New Orleans, October 1977, pp. 493-494.

8. Wharton RS, Sievenpiper TS, Wilson AI, Rice SA and Mazze RI: Developmental toxicity of methoxyflurane in mice. Teratology 17:41A, 1978.

9. Rice SA, Sbordone L and Mazze RI: Induction of in vitro anesthetic defluorination by isoniazid. Pharmacologist 20:258, 1978.

10. Sievenpiper TS, McClendon FD, Mazze RI and Rice SA: Renal effects and metabolism of enflurane in Fischer 344 rats with pre-existing renal insufficiency. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 303-304.

11. Wharton RS, Sievenpiper TS, Rice SA and Mazze RI: Developmental toxicity of methoxyflurane in Swiss/ICR mice. Abstracts of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 421-422.

12. Rice SA and Mazze RI: The induction of microsomal anesthetic defluorination by phenytoin. Abstract of Scientific Papers, American Society of Anesthesiologists Annual Meeting, Chicago, October 1978, pp. 571-572.

13. Rice SA, Sbordone L and Mazze RI: The induction of in vitro anesthetic defluorination by isoniazid. Abstracts of Scientific Papers, American Society of Anesthesiologists, Annual Meeting, Chicago, October 1978, pp. 573-574.

14. Rice SA, Maze M, Mazze RI and Kosek J: Hepatotoxicity following halothane anesthesia in isoniazid treated rats. Fed Proc 38:683, 1979.

15. Wharton RS, Wilson AI, Rice SA and Mazze RI: Teratogenicity of enflurane in Swiss/ICR mice. Teratology 19:53A, 1979.

16. Rice SA and Fish MP: Effects of isoniazid metabolites on the rate of hepatic microsomal defluorination of volatile florinated ether anesthetics. Abstract of Papers, Fourth International Symposium on Microsomes and Drug Oxidations, Ann Arbor, Michigan, July 1979, p. 111.

17. Rice SA, Baden JM and Mazze RI: Serum and urinary F^- levels following exposure to deuterated methoxyflurane (d4-MOF). Pharmacologist 21:214, 1979.

18. Fish MP and Rice SA: Isoniazid metabolites and anesthetic metabolism. Anesthesiology 51:S256, 1979.

19. Fish KJ, Sievenpiper TS, Rice SA and Mazze RI: Enflurane and gentamicin in chronic renal failure. Anesthesiology 51:S262, 1979.

20. Baden JM, Rice SA, Denson DD and Mazze RI: Deuterated methoxyflurane (d_4-MOF) Anesthesia. Anesthesiology 51:S263, 1979.

21. Rice SA and Mazze RI: Serum and urinary F^- levels and renal function following enflurane anesthesia in isoniazid treated rats. Fed Proc 39:998, 1980.

22. Baden JM, Rice SA and Mazze RI: Deuterated methoxyflurane anesthesia, No. 0028. World Conference on Clinical Pharmacology and Therapeutics, London, England, August 8, 1980.

23. Rice SA, Sievenpiper TS and Mazze RI: In vitro anesthetic defluorination and in vivo liver function in Fischer 344 rats with chronic renal failure. Anesthesiology 53:S253, 1980.

24. Rice SA and Mazze RI: Chronic intermittent exposure of Swiss W/ICR mice to N_2O. No. 457. Presented at Eighth International Congress of Pharmacology, IUPHAR, Tokyo, Japan, July 19-24, 1981.

25. Mazze RI, Rice SA, Baden JM and Wilson AI: Effects of nitrous oxide on fetal development and male fertility in mice. Anesthesiology 55:A188, 1981.

26. Rice SA: Developmental effects of N_2O exposure during organogenesis. Pharmacologist 24:163, 1982.

27. Rice SA, Mazze RI, Wyrobek AJ, Felton JS, Brodsky JB and Baden JM: Germ cell studies in mice after prolonged exposure to nitrous oxide. Anesthesiology 57:A368, 1982.

28. Fish KJ and Rice SA: Halothane inhibits metabolism of enflurane. Anesthesiology 57:A241, 1982.

29. Baden JM, Kelley M, Rice SA and Mazze RI: Decreased thymidine production in cells exposed to nitrous oxide. Anesthesiology 57:A243, 1982.

30. Rice SA, Millan DP and West J: The behavioral effects of perinatal methimazole exposure in Swiss Webster mice. Teratology 27:71A, 1983.

31. Mazze RI, Wilson AI, Rice SA and Baden JM: Reproduction and fetal development in rats exposed to nitrous oxide. Anesthesiology 59:A373, 1983.

32. Mazze RI, Wilson AI, Rice SA and Baden JM: Effects of isoflurane on reproduction and fetal development in mice. Anesth Analg 63:249, 1984.

33. Rice SA, Mazze RI and Baden JM: Reproductive and teratogenic effects of isoflurane in Swiss Webster mice. Teratology 29:54A, 1984.

34. Rice SA: Behavioral effects of prenatal exposure to nitrous oxide (N_2O) in Swiss Webster mice. Teratology 29:7B, 1984.

35. Rice SA: Behavioral effects of prenatal exposure to nitrous oxide (N_2O) in Swiss Webster mice. Teratology No. 1797. Presented at the Ninth International Congress of Pharmacology, IUPHAR, London, England, July 1984.

36. Rice SA and Fish KJ: Comparison of the cytochrome P-450 mediated metabolism of fluorinated inhalation anesthetics and resultant nephrotoxicity in obese and normal male Fischer 344 rats. Presented at the Sixth International Symposium on Microsomes and Drug Oxidations, Brighton, England, August 5-10, 1984. Abstract No. P.3/53.

37. Rice SA and Fish KJ: Metabolism and renal function in non-obese and obese rats exposed to enflurane or isoflurane. Anesthesiology 61:A264, 1984.

38. Fish KJ, Rice SA and Weissman DB: Halothane inhibits metabolism of enflurane in surgical patients. Anesthesiology 61:A268, 1984.

39. Rice SA and Steffey EP: Metabolism of halothane and isoflurane in horses. Veterinary Surgery 14:76, 1985.

40. Serra MT and Rice SA: Relationship of isoniazid (INH) acetylation rate and defluorination rates of enflurane (ENF), isoflurane (ISO) and methoxyflurane (MOF). Fed Proc 44:1253, 1985.

41. Wang S-L, Rice SA and Serra MT: Identification of defluorination activity in purified rat liver cytosol glutathione S-transferases (GTs). Fed Proc 44:1821, 1985.

42. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden J: Reproductive effects of the inhalation anesthetics, halothane, enflurane, isoflurane and nitrous oxide. Teratology 31:55A, 1985.

43. Rice SA and Millan DP: Behavioral effects of in utero nitrous oxide (N_2O) exposure in adult SW mice. Teratology 31:6B, 1985.

44. Biermann JS, Rice SA and Fish KJ: Altered metabolism of halothane in obese Fischer 344 rats. Anesthesiology 63:A542, 1985.

45. Mazze RI, Fujinaga M, Rice SA, Harris SB and Baden JM: Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. Anesthesiology 63:A439, 1985.

46. Rice SA and Steffey EP: Metabolism of three inhaled anesthetics by cow, dog, lamb and rat hepatic microsomes. Proceedings, p. 164. Presented at the 2nd International Congress Veterinary Anesthesiologists, Sacramento, California, October 1985.

47. Rice SA: Prenatal N_2O exposure alters startle reflex in adult mice. Toxicologist 6:75, 1986.

48. Rice SA: Behavioral effects of in utero isoflurane exposure in young SW mice. Teratology 33:100C, 1986.

49. Margary J, Rice SA and Fish KJ: Propylene glycol and amidate^R inhibit enflurane metabolism in Fischer 344 rats. Anesthesiology 61:A249, 1986.

50. Rice SA and Wang S-L: Defluorination by hepatic glutathione S-transferases? Toxicologist 7:217, 1987.

51. Rice SA: Effects of prenatal isoflurane exposure on postweaning activity and reactivity in SW mice. Teratology 35:10B, 1987.

52. Rice SA: Effects of prenatal isoflurane exposure on postweaning activity and reactivity in SW mice. 10th International Congress of Pharmacology, IUPHAR, Sydney, Australia, August 1987.

53. Rice SA and Fish KJ: Anesthetic considerations for drug metabolism studies in laboratory animals. J Lab Animal Med 37:520, 1987.

54. Fish KJ, Rice SA and Margary J: Isoflurane metabolism in obese rats with chronic renal insufficiency. Anesthesiology 67:A303, 1987.

55. Baer ER, Pearl RG, Siegel LC and Rice SA: Longitudinal distribution of pulmonary vascular resistance during endotoxin-induced pulmonary hypertension in sheep. Anesthesiology 69:A860, 1988.

56. Pearl RG and Rice SA: Thromboxane mediation of propylene glycol-induced pulmonary hypertension in sheep. Anesthesiology 69:A861, 1988.

57. Rice SA and Ebling WF: F^- disposition in obese and non-obese F344 rats. Toxicologist 9:88, 1989.

58. Rice SA: Startle reflex reactivity of rats perinatally treated with methimazole or prenatally exposed to N_2O. Teratology, 39:508, 1989.

59. Rice SA and Carughi A: Embryonic and fetal folates following N_2O exposure. Toxicologist, 10:40, 1990.

60. Carughi A and Rice SA: Embryonic and fetal folates in N_2O-exposed rats. FASEB Journal, 4:A873, 1990.

61. Rice SA, Carughi A and Serra MT: Startle hyperreactivity following repeated prenatal N_2O exposure in SD rats. Teratology 41:619, 1990.

62. Fish KJ, Rice SA and Peterson KL: Renal function following cyclosporine and enflurane. Anesthesiology 73:A361, 1990.

63. Rice SA, Carughi A and Serra MT: Fetal brain catecholamines and indoleamines following repeated nitrous oxide exposure. Society for Neuroscience Abstracts 16:1118, 1990.

64. Rice SA, Phillips SJ, Allen KL, Gordon GR and Tyson CA: Acute effects of cyclosporin A and isoflurane anesthesia in a model of renal transplantation. Toxicologist 12:224, 1992.

65. Ray RM, Donelson A, Rice S and Kalinowski A: Fatal motor vehicle accidents: The role of drowsiness, fatigue & antihistamines with such effects. To be presented at the 12th World Congress of the International Association for Accident and Traffic Medicine, 7th Nordic Congress on Traffic Medicine, Helsinki, June 23-25, 1992.

BOOK CHAPTERS AND THESIS

1. Rice SA: The Role of Histamine in the Pathogenesis of Pulmonary Edema and in the Development of Tolerance to Thiocarbamide Compounds. Ph.D. Thesis, University of Michigan Microfilms, Ann Arbor, Michigan, 1977.

2. Baden JM and Rice SA: Metabolism and Toxicity of Inhaled Anesthetics. In: Anesthesia, Vol 1, pp. 383-424. Miller RD (Ed.). Churchill Livingstone Inc., New York, 1981.

3. Rice SA: Behavioral Toxicity of Inhalational Anaesthetic Agents. In: Clinics in Anaesthesiology, Vol 1, No 2, pp. 507-519. Mazze RI (Ed.). W.B. Saunders Company Ltd., London, 1983.

4. Rice SA and Pellegrini MJ: Basic Principles of Teratology. In: The Pregnant Surgical Patient, pp. 1-28. Baden JM and Brodsky JB (Eds.). Futura Publishing Company, Inc., New York, 1985.

5. Rice SA and Pellegrini MJ: Teratogenicity of Fixed Agents. In: The Pregnant Surgical Patient, pp. 53-82. Baden JM and Brodsky JB (Eds.). Futura Publishing Company, Inc., New York, 1985.

6. Baden JM and Rice SA: Metabolism and Toxicity of Inhaled Anesthetics. In: Anesthesia, Vol 1, pp. 701-744. Miller RD (Ed.). Churchill Livingstone Inc., New York, Second Edition, 1986.

7. Baden JM and Rice SA: Metabolism and Toxicity. In: Anesthesia, Vol 1, pp. 135-170. Miller, RD (Ed.). Churchill Livingstone Inc., New York, Third Edition, 1990.

8 Rice SA: Anaesthesia in Pregnancy and the Fetus: Toxicological Aspects. In: Effects on the Baby of Maternal Analgesia and Anaesthesia. pp 88-107. Reynolds F (Ed.). W.B. Saunders Company Limited, London, 1993.

9. Baden JM and Rice SA: Metabolism and Toxicity. In: Anesthesia, Vol 1, Chapter 8. pp 157-183. Miller, RD (Ed.). Churchill Livingstone Inc., New York, Fourth Edition, 1994 (in press).

10. Rice SA and Fish KJ: Anesthetic Toxicity: An Overview. In: Anesthetic Toxicity. pp 1-7. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

11. Rice SA: Reproductive and Develpomental Toxicity of Anesthetics in Animals. In: Anesthetic Toxicity. pp 157-174. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

12. Ebi K and Rice SA: Reproductive and Develpomental Toxicity of Anesthetics in Humans. In: Anesthetic Toxicity. pp 175-198. Rice SA and Fish KJ (Eds.). Raven Press, Ltd., New York, 1994.

13. Rice SA: Developmental Neurotoxicity Tests - Data Evaluation. In: Handbook of Developmental Toxicity. Hood RD (Ed.). CRC Press, Inc., Boca Raton, Florida (in press).

BOOK

Rice SA and Fish KJ (Editors): Anesthetic Toxicity, Raven Press, Ltd., New York, 1994, 306 pp.

APPENDIX II

TABLES 1-5

Table 1: Comparison of Particulate Phases of Mainstream Smoke, Sidestream Smoke, and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Particle Number (no./cigarette)	10.5×10^{12}[b]	3.5×10^{12}[b]		U.S. DHHS, 1986
Particle Concentration (no. particles/cm^3)	5.3×10^9			U.S. DHHS, 1986
	10^9-10^{10}		~1.5×10^5	Rodgman, 1992
	~1×10^{10}			Guerin, 1987
Particle Concentration ($\mu g/m^3$)			18.4-64	U.S. EPA, 1992
			22.3 (7-77)[d,f]	Holcomb, 1993
			45.9 (ND-240)[d,g]	
			49.5 (17-212)[e,f]	
			67.7 (12-2700)[e,g]	
			103.7[e,h]	
			131.5 (ND-685)[e,i]	
			<120-986	IARC, 1986
			460[j,r]	Lofroth et al., 1989
			320-470[h,s]	
			>1500[k,n]	

Table 1 (con't): Comparison of Particulate Phases of Mainstream Smoke, Sidestream Smoke, and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Total Particle Weight (mg/cigarette)	15-40[p]	19.5-52[l]		U.S. DHHS, 1986
	15-40[b,c,p]	19.5-52[l,o,p]		
		16.9[m]		U.S. EPA, 1992
		16-36[n]		
		20-23[n]		
		19.8-23.4[q]		Guerin, 1987
Particle Size (μm)	0.1-1.0	0.01-0.80		U.S. DHHS, 1986 & NRC, 1986
Particle Mean Diameter (μm)	0.3-0.4[b]	0.2	0.15-0.20	Rodgman, 1992
	0.40[b]	0.32[b]		NRC, 1986 & U.S. DHHS, 1986

[a] Data from indicated sources
[b] Fresh & undiluted smoke
[c] Total particulate matter
[d] Nonsmoking area; ( ) = range
[e] Smoking area; ( ) = range
[f] Homes
[g] Office/public places
[h] Bars
[i] Restaurants
[j] Average
[k] Peak
[l] Calculated from SSS/MSS = 1.3
[m] Filtered 1R4F Kentucky Reference Cigarette
[n] Commercial cigarette
[o] Diluted SSS
[p] Unfiltered cigarette
[q] U.S. type cigarette blend
[r] 2R1 reference cigarette
[s] Total respirable particles

Table 2: Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS[a]

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
Carbon monoxide (CO)[b]	mg/cig	mg/cig	mg/m^3	
			1.79-4.76	Lofroth et al., 1989
	10-23			IARC, 1986
	10-23			NRC, 1986
	14-23[e]	26.8-61		Hoffmann & Hecht, 1990
	10-23		25-108.1[d]	U.S. EPA, 1992
	54			
	11.3[g]	54.1	2.69	RJR, 1988
		49.6-58.1[i]		Guerin et al., 1987
			2.4[h]	Lofroth et al., 1989
			5.1-9.8[j]	
			3.4-6.9	Holcomb, 1992
			4.6	Guerin, 1992
Benzo[a]pyrene (BaP)	ηg/cig	ηg/cig	$ηg/m^3$	
	9.2[g]	147.9		RJR, 1988
			1.07	Guerin, 1992
	20-40		1.7-460	NRC, 1986
	20-40[e]	40-70	3.3-23.4	Hoffmann & Hecht, 1990
	20-40		50-140[k]	U.S. DHHS, 1986
			0.25-760	IARC, 1986
	5-78	25-199	2.8-760	IARC, 1983

Table 2 (con't): Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
4-(N-nitrosomethyl-amino)-1-(3-pyridyl)-1-butanone (NNK)	ηg/cig	ηg/cig	ηg/m^3	
			< 1-3	Guerin, 1992
		400	1.9-29.3	U.S. EPA, 1992
	80-770	190[f]		IARC, 1986
	84.0	419		RJR, 1988
	80-770[e]	200-1400		Hoffmann & Hecht, 1990
	100-1000	100-4000[n]		U.S. DHHS, 1986; NRC, 1986
N-nitrosonornicotine (NNN)	ηg/cig	ηg/cig	ηg/m^3	
			< 1-3	Guerin, 1992
			1.8-22.8	U.S. EPA, 1992
	120-3700	150-1700		Hoffmann & Hecht, 1990
	101[g]	171		RJR, 1988
	200-3000	150		IARC, 1986; **NRC, 1986**
N-nitrosodimethylamine (NDMA)	ηg/cig	ηg/cig	ηg/m^3	
			1.0-10	Guerin, 1992
	10-40		20-200	NRC, 1986
	ND[g,o]	298		RJR, 1988
	2-20	736	10-240	IARC, 1986
		200-800[n]		U.S. EPA, 1992
	0.1-180	200-1040	0-240	Hoffmann & Hecht, 1990

Table 2 (con't): Comparison of Amounts of Selected Constituents of Mainstream Smoke (MSS), Sidestream Smoke (SSS), and ETS

	Mainstream Smoke	Sidestream Smoke	ETS	Reference
N-nitrosodiethylamine (NDEA)	ηg/cig	ηg/cig	ηg/m^3	
	ND-25		0-200	Hoffmann & Hecht, 1990
			< 10	Guerin, 1992
	ND			RJR, 1988
	ND-25	< 1000[p]		U.S. EPA, 1992
		200-1400		

[a] Data are complied from several sources
[b] 1 ppm = 1.15 mg/m3
[c] Cig = cigarette
[d] Calculated from SSS/MSS ratio = 2.5-4.7
[e] Unfiltered cigarette
[f] Filtered cigarette
[g] 1R4F reference cigarette
[h] 2R1 reference cigarette
[i] U.S. type blend cigarette
[j] Commercial cigarette
[k] Calculated from SSS/MSS ratio = 2.5-3.5
[m] Calculated from SSS/MSS ratio = 1-4
[n] Calculated from SSS/MSS ratio = 20-100
[o] Not detected
[p] Calculated from SSS/MSS ratio = < 40

Table 3: Species Differences in Minute Volume per Unit Lung Weight[a]

Species	minute volume (l/min)	lung weight (g)	minute volume/g lung (l/min-g)
mouse	0.021	0.20	0.105
rat	0.16	1.6	0.10
human (male)	6.4	1065.0	0.006

[a] Adapted from Table 6, Phalen (1984c)

Table 4: *In Vivo* Inhalation Studies of ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (F344)	M/F	4	10	5	90 d	Histopathology of: nasal cavity, larynx, trachea, lungs, liver, kidneys, adrenals, brain, pituitary, bladder, heart, thyroid, parathyroid, thymus, mammary gland, testes, ovaries	Hyperplasia & squamous metaplasia of epithelium of dorsal nasal turbinate of rats No significant effects in the other organs examined	von Meyerinck et al., 1989
					30 d recovery following 90 d exposure		Histopathological changes partially receded	
					60 d & 90 d recovery following 90 d exposure		Histopathological changes completely reversed	
Hamster (Syrian gold)	M/F				Same exposures as rats		No significant effects	
Rat (S/D)	M/F	0.1 1 10	6	5	14 d	Histopathology of: nasal passages, larynx, trachea, conducting airways, deep lung, heart, lymph nodes	Slight to mild epithelial hyperplasia & inflammation in rostral part of nasal cavity in 10 mg/m^3 group	Coggins et al., 1992
					14 d recovery following 14 d exposure		Histopathological changes were reversible	

Table 4 (con't): *In Vivo* Inhalation Studies of ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D)	M	0.1 1 10	6	5	4 d	Histopathology of: nasal passages, larynx, trachea, conducting airways, deep lung, heart, lymph nodes	Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	Coggins et al., 1993
					28 d		Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	
					90 d		Slight to mild epithelial hyperplasia in rostral cavity at 10 mg/m^3	
					90 d recovery following 90 d exposure		No significant histopathological changes compared to control	
Rat (S/D)	M	2[a] 6[b]	7	7	90 d	Histopathology of: nose, larynx, vocal cords, trachea & lungs	Laryngeal epithelium: slight hyperplasia & slight squamous metaplasia Cuboidal epithelium: hyperplasia Pseudostratified epithelium: squamous metaplasia Vocal cord squamous epithelium: hyperplasia All histopathological changes occurred at 6 mg/m^3	Teredesai & Pruhs, 1994
					90 d	Thickness of: laryngeal epithelium and vocal cords	6 mg/m^3: +19% larynx & +32% lower medial surface of vocal cords; statistically different from control	
					21 d recovery following 90 d exposure	Histopathology of: nose, larynx, vocal cords, trachea & lungs	Histopathological changes were reversed except vocal cord hyperplasia	
Hamster (Syrian gold)	M				Same exposures as rats		No significant effects	

[a] (2 µg/L)
[b] (6 µg/L)

Table 5: *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Mouse (NMRI)	M	1 cig 2 cig 3 cig 4 cig	NA[c]	every 2 hr	NA	Clastogenesis @ 30 hr: micronuclei in femoral bone marrow polychromatic erythrocytes (PCE)	Clastogenesis (micronuclei/1000 cells): ~3.2 (1 cig); ~3.5 (2 cig); ~4.0 (3 cig); ~3.5 (4 cig) vs. ~1.75 (control) All significant increases from control	Mohtasham-ipur et al., 1987
Rat (S/D)	M & F	0.1 1 10	6	7	7 d	Chromosomal aberrations in alveolar macrophages	No significant differences from control	Lee et al., 1992
						DNA adduct formation[b]: lung, heart, larynx, liver	Lung (adducts/10^9 nucleotides): ~ 8.2 (10 mg/mg^3) vs. ~3.75 (control) Other organs & doses were not significantly different from control	
					14 d		Lung: ~8.7 (10 mg/mg^3) vs. ~3.75 (control) Heart: ~5.7 (10 mg/mg^3) vs. ~2.0 (control)	
					14 d recovery following 14 d exposure		Lung: ~8.0 (10 mg/mg^3) vs. ~3.75 (control) Heart: ~6.0 (10 mg/mg^3) vs. ~2.0 (control)	
Rat (S/D)	M	0.1 1 10	6	5	28 d	DNA adduct formation: lung, heart, larynx, liver & bladder	Lung (adducts/10^9 nucleotides): ~20 (10 mg/mg^3) vs. ~5 (control) Heart: ~7.5 (10 mg/mg^3) vs. ~3.0 (control) Larynx: ~10 (10 mg/mg^3) vs. ~4.5 (control) Liver: no significant differences from control Bladder: not measured	Lee et al., 1993

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D) (con't)	M	0.1 1 10	6	5	90 d		Lung: ~24 (10 mg/mg^3) vs. ~5 (control) Heart: ~17.5 (10 mg/mg^3) vs. ~5.0 (control) Larynx: ~27.5 (10 mg/mg^3) vs. ~4.5 (control Liver, bladder: not significantly different from control	Lee et al., 1993
					90 d recovery following 90 d exposure		Lung: ~15 (10 mg/mg^3) vs. ~6 (control) Heart: ~12.5 (10 mg/mg^3) group vs. ~4.5 (control) Larynx: ~13.0 (10 mg/mg^3) vs. ~5.0 (control) Liver, bladder: not significantly different from control	
Hamster (Syrian gold)	M	1.03	6	7 d	1 wk	Cell proliferation[c]: lung parenchyma, intrapulmonary airways, trachea, nasal passages	Nasal septum: cumulative labeling index 17.5% vs. 13% (control) Labeling indexes for all other sites were not significantly different from control	Witschi, et al., 1994
					1 wk recovery following 1 wk exposure		Terminal bronchioles: 11% vs. 8% (control) Maxillary turbinates: 14.5% vs. 20% (control)	
					2 wk exposure		Labeling indexes not significantly different from control	
					2 wk exposure followed by 1 wk recovery		Maxillary turbinates: 15% vs. 19.5% (control)	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Hamster (S. gold) (con't)	M	1.03	6	7 d	3 wk exposure		Labeling indexes not significantly different from control	Witschi, et al., 1994
					1 wk recovery following 3 wk exposure		Labeling indexes not significantly different from control	
Mouse (A/J)	M	1	6	5	1 d	Cell proliferation[c]: airways & lung parenchyma	No significant effects	Rajini et al., 1994
					3 d		Nasal epithelium of large airways: labeling index 8% vs. 3.5% (control) Terminal bronchioles: 7% vs. 2% (control)	
					5 d		Nasal epithelium of large airways: 11% vs. 5 % (control) Terminal bronchioles: 13% vs. 3% (control)	
Mouse (C57BL /6)	M	1	6	5	1 d 3 d 5 d	Cell proliferation[c]: airways & pulmonary parenchyma	No significant differences from control.	Rajini et al., 1994
Rat (S/D)	M	0.1 1 10	6	5	5 d	Cell proliferation[c]: nasal cavity, ventral larynx, trachea, & lung (bronchial, bronchioles, alveoli)	Cells labeled/1.5 mm nasal cuboidal: 88.5 in (1 mg/m^3), 168.1 (10 mg/m^3) vs. 40.5 (control) Cells labeled/1.0 mm nasal respiratory region: 26.1 (1 mg/m^3), 23.7 (10 mg/m^3) vs. 22.3 (control) Cells labeled/1.0 mm nasal squamous tissue: 299.5 (1 mg/m^3), 259 (10 mg/m) vs. 291.9 (control)	Ayres et al., 1995
					28 d		No significant differences from control	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D) (con't)	M	0.1 1 10	6	5	90 d	Cell proliferation[c]: nasal cavity, ventral larynx, trachea, lung (bronchial, bronchioles, alveoli)	Increased DNA synthesis in cuboidal & respiratory epithelium in most rostral portion of nasal cavity	Ayres et al., 1995
					90 d recovery following 90 d exposure		No increases in DNA synthesis in any tissue	
Mouse (A/J)	M	4	6	5	1 wk 2 wk 3 wk	Cell proliferation[c]: nasal cavity, trachea, lung	Few significant effects in lung Increase in cell proliferation in nasal passageways & somewhat in airways	Witschi et al., 1995
					4 wk		No significant effect	
					6 wk		No significant effects	
					9 wk		Some increase in cell proliferation in nasal & maxillary turbinates	
					16 wk		Some increase in cell proliferation in nasal & maxillary turbinates	

Table 5 (con't): *In Vitro* Mechanistic Studies Following Inhalation Exposure to ADSS

		Exposure Conditions						
Species (Strain)	**Sex**	**Particles (mg/m^3)**	**Duration (hr/d)**	**Frequency (d/wk)**	**Period of Exposure**	**Assessment & Site**	**Results**	**Reference**
Rat (S/D)	neo-nate	0	NA	NA	birth	Lung P4501A1[d] & P4502B1[e]	No activity detected	Gebre-michael et al., 1995
		1.0	6	5	7 d		Lung P4501A1: detected in exposed group Lung P4502B1: detected, not different	
					14 d		Lung P450 1A1: ~9.5 pmol/mg/min (exposed) vs. 2.04 pmol/mg/min (control) Lung P4502B1: no significant differences	
					21 d		Lung P450 1A1: ~25 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
					50 d		Lung P450 1A1: ~37.5 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
					100 d		Lung P450 1A1: ~32.5 pmol/mg/min (exposed) vs. 9.77 pmol/mg/min (control) Lung P4502B1: no significant differences	
Rat (S/D)	M	1.0	6	4 d	4 d		Lung P4501A1 levels similar to those measured in 100 d exposure group Lung P4502B1: no significant differences	

[a] Not applicable
[b] P^{32} Post-labeling is used to quantify DNA adduct formation.
[c] Incorporation of BrdU is used to measure replicative DNA synthesis, a measure of cell proliferation.
[d] Ethoxyresorufin-O-dealkylase (EROD) activity is a measures of cytochrome P4501A1
[e] Pentoxyresorufin-O-dealkylase (PROD) activity is a measure of cytochrome P4502B1

COMMENTS OF THE

R. J. REYNOLDS TOBACCO COMPANY

on

Health Effects of Exposure to Environmental Tobacco Smoke

Final Draft, February 1997

by the

California Environmental Protection Agency

May 5, 1997

TABLE OF CONTENTS

I. INTRODUCTION

The R. J. Reynolds Tobacco Company ("RJR") would like to thank the California Environmental Protection Agency ("Cal/EPA") Office of Environmental Health Hazard Assessment ("OEHHA") for the opportunity to comment on the document titled Health Effects of Environmental Tobacco Smoke (February 1997) (Final Draft Report) (the "1997 Draft"). RJR hopes that these comments will assist the Agency in its efforts to accurately describe the current state of knowledge regarding environmental tobacco smoke ("ETS"). In accordance with the guidance offered in Cal/EPA's notice to interested parties, RJR's comments presented here address only what RJR believes are some of the new issues or new information not previously considered by Cal/EPA. Consequently, the Agency should not infer that the absence of comment on other issues presented in the 1997 Draft necessarily implies RJR's acceptance of or agreement with the conclusions reached or data reported by Cal/EPA.

On March 7, 1997, Cal/EPA released the 1997 Draft. The 1997 Draft contains eight chapters and an appendix that address ETS and the following topics (Exposure Measurements and Prevalence, Developmental Toxicity I: Perinatal Manifestations, Developmental Toxicity II: Postnatal Manifestations, Reproductive Effects, Respiratory Health Effects, Carcinogenic Effects, Cardiovascular Effects, and Summary of Public Comments and Responses). By notice, Cal/EPA solicited public comments as part of the development of the 1997 Draft. RJR submits these comments on the 1997 Draft.

The May 1997 comments focus on the following issues: (1) the egregious inadequacy of Cal/EPA's non-response to many issues raised in RJR's comments on Cal/EPA's previous draft chapters; (2) relevant scientific information and analysis not previously considered by Cal/EPA; and (3) specific new issues raised by the scientific evidence on ETS.

II. EXECUTIVE SUMMARY

A. The Cal/EPA 1997 draft is an incomplete and inadequate assessment of ETS and health because it fails to consider and analyze all readily available and relevant scientific evidence and issues raised by public comments

It is incumbent on Cal/EPA to review and analyze all of the additional scientific evidence presented in these comments. Cal/EPA should use this additional scientific information to identify the best available evidence to answer the questions before the Agency. Cal/EPA is obligated to: 1) consider all relevant studies of ETS; 2) identify the studies that comprise the best available evidence; and 3) base any conclusions on the best available evidence. As demonstrated by these comments, the 1997 Draft does not fulfill Cal/EPA's obligations.

1. Chapter 2: Exposure Measurements and Prevalence

On October 16, 1995, RJR submitted comments ("RJR 1995 Cal/EPA Exposure Comments") on Cal/EPA's August 1995 External Review Draft titled "Environmental Tobacco Smoke: Exposure Measurements and Prevalence" ("1995 Exposure External Review Draft"). Cal/EPA's 1997 Draft includes a purported revision of the 1995 Exposure External Review Draft Chapter. Since October 16, 1995, additional studies have been published that provide new information that Cal/EPA must consider as it attempts to produce a revised chapter on ETS Exposure Measurements and Prevalence in its 1997 Draft. RJR has identified 14 additional scientific publications that Cal/EPA must consider in its analysis of ETS exposure measurements and prevalence.

2. Chapter 3: Developmental Toxicity I (Fetal Birthweight)

In Chapter 3 (Developmental Toxicity I: Perinatal Manifestations) of the 1997 Draft, Cal/EPA reviews the scientific literature regarding ETS and fetal birthweight. Cal/EPA concludes that ETS exposure is causally associated with decreased fetal birthweight. In Appendix A to these

comments, Dr. James Swauger reviews and analyzes the scientific evidence on ETS and fetal birthweight. Dr. Swauger focuses on the new information identified by Cal/EPA in the 1997 Draft and identifies new issues not previously considered by Cal/EPA. These comments demonstrate that the available scientific evidence is insufficient to support a causal conclusion.

Appendix A identifies the following issues that Cal/EPA must address in its analysis of fetal birthweight and ETS:

- The published literature on ETS and fetal birthweight is weak and inconsistent.
- The epidemiologic studies do not consistently control for important confounders in studies of fetal birthweight. These confounders include: gestational age, race, birth order, education level, maternal age, paternal smoking behavior, maternal weight, weight gain during pregnancy, maternal height, dietary patterns, smoking status, alcohol use, caffeine intake, social status, fetal sex, and gravidity.
- The epidemiologic studies do not sufficiently control for smoking status misclassification bias.
- Prior knowledge of birth outcome can result in recall bias due to selective reporting of smoking behavior and ETS exposure by new mothers.
- None of the epidemiologic studies attempt to quantitatively measure ETS exposures.
- The epidemiologic studies of ETS and fetal birthweight show an absence of a dose-response relationship.
- Animal models have provided limited and inconsistent results.

3. Chapter 4: Developmental Toxicity II (SIDS)

In Chapter 4 (Developmental Toxicity II: Postnatal Manifestations) of the 1997 Draft, Cal/EPA reviews the scientific literature regarding ETS and SIDS. Cal/EPA concludes that post-natal ETS exposure is causally associated with sudden infant death syndrome ("SIDS"). In Appendix A to these comments, Dr. James Swauger reviews and analyzes the scientific evidence on post-natal ETS exposure and SIDS. Dr. Swauger's comments focus on the new information

identified by Cal/EPA in the 1997 Draft and identifies new issues not previously considered by Cal/EPA. Dr. Swauger demonstrates that the scientific evidence cited by Cal/EPA is insufficient to support this conclusion.

Appendix A identifies the following issues that Cal/EPA must address in its analysis of SIDS and ETS:

- The epidemiologic studies of ETS and SIDS are weak and inconsistent.
- The epidemiologic studies do not consistently control for important confounders in epidemiologic studies of SIDS. These confounders include: region, time of day, marital status of mother, maternal smoking, season, maternal age, parity, ethnic group, fetal birthweight, fetal age, breast feeding, social status, sleep position, and bed sharing.
- The epidemiologic studies do not adequately control for misclassification of smoking status.
- Two epidemiologic studies cited by Cal/EPA (Milerad *et al.*, 1994 and Haglund *et al.*, 1995) did not distinguish between active maternal smoking during pregnancy and postnatal ETS exposure. Therefore, the methodology of the studies does not allow an appropriate examination of postnatal ETS exposure and SIDS.
- None of the epidemiologic studies attempt to quantitatively measure ETS exposures.
- The epidemiologic studies of ETS and SIDS do not show a dose-response relationship.
- Three epidemiologic studies cited by Cal/EPA (Mitchell *et al.*, 1995, Klonoff-Cohen *et al.*, 1995, and Blair *et al.*, 1996) collected information regarding smoking behavior (i.e., exposure) following the death of the infant, in some cases years later. Prior knowledge of the outcome may have resulted selective recall bias in reporting smoking behavior and ETS exposures.

4. Chapter 6: Respiratory Health Effects

Chapter 6 (Respiratory Health Effects) of Cal/EPA's 1997 Draft purports to review "a substantial body of literature" that addresses acute and reversible irritative effects and ETS. In Appendix B to these comments, Dr. James C. Walker illustrates major methodologic shortcomings

in Cal/EPA's review of the available data concerning ETS and sensory irritation and annoyance. Dr. Walker's comments point out the following deficiencies that must be addressed by Cal/EPA:

- Cal/EPA should use operational definitions to lessen the ambiguity of using words like "irritation" that have multiple meanings.

- Cal/EPA should pay close attention to real-world ETS concentrations and exposures, and analyze whether the hypothesized effects asserted by Cal/EPA have been shown to occur in environmentally realistic ETS conditions.

- Cal/EPA presents a great deal of information irrelevant to a scientific discussion on the issue of perceived odor or irritation and ETS.

- Cal/EPA should delete phrases like "mucous membrane symptom" that add confusion and are not defined.

- Cal/EPA should eliminate or adequately characterize the results of the experimental studies that have not been replicated (e.g., Ahlstrom *et al.*, 1987).

- Cal/EPA must include recently published studies that are directly relevant to sensory impact and ETS, which were conducted in representative current indoor air environments.

- Cal/EPA relies excessively on second or third-order reviews and opinions that are of dubious quality. Cal/EPA should focus on the peer-reviewed literature and original research data.

A proper analysis of these issues and new information submitted by RJR will invalidate Cal/EPA's conclusion that nonsmokers can be protected against sensory irritation and odor annoyance only through a prohibition of smoking or extreme engineering measures.

5. Chapter 7: Carcinogenic Effects

On April 1, 1996, R. J. Reynolds Tobacco Company ("RJR") submitted comments ("RJR 1996 Cal/EPA Lung Cancer Comments") on the California Environmental Protection Agency's ("Cal/EPA") January 1996 External Review Draft, titled "Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer" ("Lung Cancer External Review

Draft"). Cal/EPA's 1997 Draft includes a purported revision of the 1996 Lung Cancer External Review Draft. Since January 1996, additional scientific evidence has been published that provides new information that Cal/EPA must consider as it attempts to produce a revised chapter on ETS and Lung Cancer. RJR submits 17 new publications that Cal/EPA must consider as it revises Chapter 7 of the 1997 Draft. The analysis contained in Chapter 7 of the 1997 draft regarding lung cancer is incomplete and uninformed by important new findings.

6. Chapter 8: Cardiovascular Effects

Chapter 8 (Cardiovascular Health Effects) of Cal/EPA's 1997 Draft reviews 17 epidemiologic studies of ETS and cardiovascular disease. In Appendix C to these comments, Dr. Carr Smith and Patricia O. DeLuca illustrate a major methodologic limitation of the epidemiologic studies of cardiovascular disease and ETS. All 17 epidemiologic studies of ETS and cardiovascular disease suffer from a significant methodologic deficiency of selection/diagnostic bias that creates variability and uncertainty in the reported results. These epidemiologic studies depend on inadequate measures of cardiovascular disease incidence by relying on death certificates, medical records, and interviews (patients and/or next-of-kin) to determine the cause of death of study subjects. An autopsy is the only method of classification that can accurately establish a cause of death. Because inaccurate diagnoses are more likely to occur in patients who are married to smokers (Cal/EPA's chosen surrogate for spousal ETS exposed subjects), the results reported in the 17 epidemiologic studies are subject to this selection/diagnostic bias that introduces variability and uncertainty in the results.

B. Cal/EPA breached its obligation to consider and address relevant public comments on its prior draft chapters

In the 1997 Draft, Cal/EPA has included an appendix that purports to respond to the issues raised by public comments to its previous draft chapters, including issues raised by RJR. On October 16, 1995, RJR submitted comments in response to Cal/EPA's external review draft chapter titled Exposure Measurements and Prevalence -- External Review Draft Chapter (August 1995) (the "1995 Exposure ~~Draft Chapter~~"). ~~However~~, Cal/EPA's appendix failed to respond to many of the issues raised in RJR's 1995 comments, and when Cal/EPA did attempt to respond, the responses were inadequate. On April 1, 1996, RJR submitted comments in response to Cal/EPA's external review draft chapter titled Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer -- External Review Draft Chapter (January 1996) (the "1996 Lung Cancer Draft Chapter"). Cal/EPA failed to acknowledge or address RJR's 1996 comments in the appendix of the 1997 Draft.

In these comments, RJR identifies each of the issues presented in RJR's prior comments that were either ignored completely or were not considered in the 1997 Draft. Cal/EPA must address each of these issues in its analysis of the available scientific information on ETS and health.

1. RJR's Comments on the 1996 Lung Cancer External Review Draft

Chapter 7 of Cal/EPA's 1997 Draft relies on the U.S. EPA (1992) report as the benchmark for its assessment of ETS and lung cancer risk. The conclusions reached in the U.S. EPA report are misleading and based on flawed and deficient analyses. Because of the numerous deficiencies in the U.S. EPA 1992 report, Cal/EPA should not rely on the conclusions or the analyses contained in that report as a benchmark for assessing ETS and lung cancer. Among the deficiencies of the U.S. EPA report that make it an inappropriate benchmark are:

- U.S. EPA ignored the fundamental problems inherent in interpreting the weak and inconsistent associations reported in the ETS and lung cancer studies.

- U.S. EPA incorrectly analyzed confounding in the epidemiologic studies by employing an improper definition of confounding, ignoring most of the relevant data, failing to assess joint confounding, and failing to quantify the amount of bias introduced by confounding.

- U.S. EPA underestimated the influence and uncertainty of smoking status misclassification bias on the epidemiologic studies by employing a model that ignores statistical variability in the input parameters and by basing its analyses on a limited and biased subset of the then-available data.

- U.S. EPA employed less rigorous statistical analyses of key issues such as chance and dose-response because the traditional, and more rigorous, tests employed by statisticians provide results inconsistent with U.S. EPA's conclusions.

- U.S. EPA's use of meta-analysis is inappropriate because the studies are dissimilar in many ways and the model chosen by U.S. EPA underestimates statistical uncertainty. Moreover, U.S. EPA's analysis failed to include the full collection of available data and failed to adequately address confounding and other sources of bias.

- U.S. EPA's analyses violate standard epidemiologic principles and the U.S. EPA's own 1986 Guidelines for Carcinogenic Risk Assessment.

Cal/EPA's 1997 Draft reviews four post-1991 epidemiologic studies of lung cancer and ETS. The four post-1991 studies provide data that, when properly analyzed, contradict the conclusions reached by the U.S. EPA. The Brownson *et al.*, 1992 study is a negative study. The most reliable data in the Brownson study -- the direct respondents -- show no increase in risk for spousal or workplace exposure, including the highest exposure groups. The published Fontham *et al.*, 1994 report is unreliable because of flaws in the study design and interpretation. Moreover, when properly analyzed, even the flawed Fontham data show no association between reported adult ETS exposure and lung cancer risk. The Kabat *et al.*, 1995 study has important design improvements over the other studies. As the investigators accurately stated regarding their results,

"the pattern of odds ratios shows little indication of an association between ETS and lung cancer in non-smokers." Stockwell *et al.* reported no statistically significant risks and no risk at all for workplace exposure despite failing to address confounding and bias.

2. RJR's Comments on the 1995 Exposure External Review Draft

Cal EPA has apparently chosen to respond to the numerous criticisms of Chapter 2 by simply changing the objective of Chapter 2: "This chapter provides *background information* on the prevalence and measurement of exposure to ETS, and emphasizes investigation and monitoring methods used in epidemiological evaluations of health effects." Cal/EPA 1997 Draft, p. 2-1 (emphasis added).[1] By relegating exposure assessment to "background information" status, the Agency has perverted the science of risk assessment.

In addition, Chapter 2 of Cal/EPA's 1997 Draft contains the following deficiencies:

- Cal/EPA should not rely on mainstream smoke and sidestream smoke data to assess ETS. Cal/EPA's use of MS/SS constituent ratios conveys no information about the absolute concentration of the constituent in either MS or SS -- much less ETS.

- Cal/EPA's review of "Biologically Active Constituents of ETS" is uninformative, misleading and should be eliminated. Many of the constituents discussed by Cal/EPA have never been measured in ETS at levels found in real world environments.

- In assessing ETS exposures, Cal/EPA fails to address studies employing the best markers, *i.e.* 3-ethenylpyridine ("3-EP") for the ETS vapor phase and - in order of preference - solanesol, FPM and UVPM for the ETS particle phase. Cal/EPA also superficially limits its discussion of these markers based on its false assumption that these markers are not widely accepted.

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[1]In 1995 Cal/EPA stated that the purpose of the Exposure chapter was to focus on exposure issues relevant to the health effects addressed throughout the draft risk assessment: "This chapter summarizes available information on exposure to environmental tobacco smoke (ETS), with a focus on exposure relevant to health effects considered in other chapters of the overall ETS assessment." Cal EPA, 1995, p. v.

- Cal/EPA fails to recognize that personal monitoring is the only reliable means of determining an individual's actual ETS exposure.

- Cal/EPA relies upon ETS exposure studies that are not representative. Cal/EPA simply ignored the two recent ETS exposure studies by ORNL and Ogden *et al.*

- Cal/EPA appears to dismiss the effect of smoking status misclassification without citing any basis for this supposition.

- Cal/EPA's discussion of studies -- that for the most part present questionnaire results of persons claiming any ETS exposure for times ranging from the previous day to the previous week -- brings no scientifically useful information to a review of ETS exposure.

The Agency should revise Chapter 2 to address exposure assessment adequately.

III. NEW ISSUES AND NEW INFORMATION THAT CAL/EPA MUST CONSIDER

A. Chapter 2: Exposure Measurements and Prevalence

On October 16, 1995, RJR submitted comments ("RJR 1995 Cal/EPA Exposure Comments") on Cal/EPA's August 1995 External Review Draft titled "Environmental Tobacco Smoke: Exposure Measurements and Prevalence" ("1995 Exposure External Review Draft"). Cal/EPA's 1997 Draft includes a purported revision of the 1995 Exposure External Review Draft Chapter. Since October 16, 1995, additional studies have been published that provide new information that Cal/EPA must consider as it attempts to produce a revised chapter on ETS Exposure Measurements and Prevalence in its 1997 Draft. These comments are submitted to assist Cal/EPA in identifying and understanding the new studies. RJR has identified the following 14 additional scientific publications and has provided a brief discussion of the implications of the key information presented in the publications.

1. ***Use of a 24-Hour Recall Diary to Assess Exposure to Environmental Tobacco Smoke*, Emmons, K.M., Marcus, B.H., Abrams, D.B., Marshall, R., Novotny, T.E., Kane, M.E., Etzel, R.A.; Archives of Environmental Health, Vol. 51, No. 2, pp. 146-149, 1996.**

This study addresses the utility of 24-hour recall diaries for assessing ETS exposures:

> The purpose of the study was to evaluate the relationship between salivary cotinine concentrations and ETS exposure . . . estimated by a 24-hour recall diary. . . . Given that exposure data from the 24-h recall diary had a relatively weak association with cotinine levels, its use is not recommended.

Emmons *et al.*, 1996, pp. 147-148. As demonstrated in RJR's 1995 Cal/EPA Exposure Comments, exposure is the product of duration and concentration (or level).[2] The 24-hour diary analyzed by Emmons *et al.*, however, was capable of providing information about only the duration component. Emmons *et al.* state that the diary was divided into 1-hour intervals and that "[f]or each interval, [they] asked participants whether they were exposed indoors to anyone who was smoking." Emmons *et al.*, 1996, p. 147. This method provides no information relating to ETS levels that existed during the 1-hour intervals. Moreover, Emmons *et al.* concede that reliance on these 1-hour intervals also hindered their ability to assess accurately the duration component of exposure:

> It should be noted that estimated exposure recorded in the 24-hour diary was based on one-hour intervals; therefore, the actual length of time a volunteer may have been exposed to ETS could not be computed.

Emmons *et al.*, 1996, p. 148. In summary, the 24-hour recall diary failed to provide any measure of concentration or any reliable measure of duration. This method could not provide reliable information on the potential duration of exposure, much less ETS exposure.

Emmons *et al.* found no statistically significant correlation between the duration data of the 24-hour recall diary and concentrations of salivary cotinine. Emmons *et al.*, 1996, p. 148 (reporting a statistically non-significant correlation of .10 (n=130)). Emmons *et al.* also recognize the weakness of relying on biologic cotinine to assess exposures: "[b]ecause of its long half life, cotinine is a marker of exposure over several days and, therefore, does not reflect exposures only during the

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[2]RJR 1995 Cal/EPA Exposure Comments, pp. i-ii, 8.

previous 24 hours." Emmons *et al.*, 1996, p. 148.[3] Emmons *et al.* conclude that the 24-hour recall diary is not reliable for assessing exposure to ETS. Emmons *et al.*, 1996, p. 148.

2. *Use of Volatile Tracers to Determine the Contribution of Environmental Tobacco Smoke to Concentrations of Volatile Organic Compounds in Smoking Environments*, Hodgson, A.T., Daisey, J.M., Mahanama, K.R.R., Ten Brinke, J.; Environment International, Vol. 22, No. 3, pp. 295-307, 1996.

Hodgson *et al.* demonstrate (1) the superiority of 3-ethenylpyridine ("3-EP") as a marker for the ETS vapor phase, and (2) that airborne nicotine is not a good marker for the ETS vapor phase.

a. 3-EP is the best marker for the ETS vapor phase[4]

Hodgson *et al.* demonstrate that 3-EP is a better marker than airborne nicotine for assessing the ETS vapor phase:

> 3-EP is probably unique to tobacco smoke in indoor environments. In the ETS chamber study referenced above [Nelson *et al.*, 1992], the ratios of 3-EP to total VOCs were much less variable than those of nicotine to total VOCs indicating that 3-EP is a better predictor of the concentrations of the gas-phase components of ETS.

Hodgson *et al.*, 1996, p. 296 (emphasis added).[5] Hodgson *et al.* demonstrate that 3-EP is superior for assessing vapor phase ETS constituents also because 3-EP has a higher emission factor than airborne nicotine and does not deposit on surfaces at higher ventilation rates.

> 3-EP was chosen as the most suitable tracer for the volatile components of ETS because of its higher emission factor and resulting airborne concentrations and the lack of evidence for significant deposition of 3-EP to surfaces in buildings at least at higher ventilation rates.

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[3]*See also*, RJR 1995 Cal/EPA Exposure Comments, pp. 19-22.

[4]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 22-23; RJR 1996 OSHA Post-Hearing Brief, p. II-14.

[5]*See also*, RJR 1995 Cal/EPA Exposure Comments, pp. 22-23; RJR 1996 OSHA Post-Hearing Brief, pp. II-7 - II-10.

Hodgson *et al.*, 1996, p. 304.

b. Nicotine is not the best marker for the ETS vapor phase[6]

Hodgson *et al.* recognize that airborne nicotine is a poor marker for the ETS vapor phase:

> Airborne nicotine has extensively been used as an ETS marker. The majority of nicotine is found in the gas-phase, but nicotine readily absorbs onto surfaces and, therefore, is not an ideal marker for the more volatile components of ETS which have significantly lower deposition rates.

Hodgson *et al.*, 1996, p. 296.[7]

c. The findings of Hodgson *et al.* regarding the contribution of ETS to VOCs are limited to designated smoking lounges

The abstract of the published paper is misleading in that it implies (mainly by omission) that the results apply generally to buildings where smoking is permitted. The full paper indicates that the results apply only to designated smoking "lounge" areas in office buildings, and do not apply to either non-smoking areas within those buildings or to general work areas where smoking is allowed. Hodgson *et al.*, 1996, p. 301.

Hodgson *et al.* obtained their air sample measurements from five office buildings. Measurements for each building were obtained by sampling a designated smoking area and two adjoining, non-smoking areas in those buildings. In four of the buildings sampled, the smoking area was an enclosed[8] smoking lounge (two had a separate outside exhaust; the other two did not). The

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[6]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 14-17; RJR 1996 OSHA Post-Hearing Brief, p. II-23.

[7]*See also* RJR 1995 Cal/EPA Exposure Comments, p. 16; RJR 1996 OSHA Post-Hearing Brief, pp. II-15, II-23.

[8]The authors did not define what constituted an "enclosed" smoking lounge.

smoking area in the fifth building sampled was a non-enclosed but well-ventilated portion of the building's cafeteria. Hodgson *et al.*, 1996, p. 301.

Concentrations of the ETS marker compounds were below the limit of detection (LODs) in **all** adjoining non-smoking areas that were sampled. Hodgson *et al.*, 1996, p. 301. Concentrations of the ETS marker compounds were below their LODs in one smoking area (which was enclosed but without outside exhaust) and near or below their LODs in the sampled cafeteria area where smoking was permitted. Hodgson *et al.*, 1996, p. 301.

Out of the fifteen areas sampled (five designated smoking areas and ten adjoining non-smoking areas), Hodgson *et al.* limited their analysis to estimating the ETS "attributable" fractions for the VOCs in only four of these areas, all of which were designated smoking areas. ETS "attributable" fractions for the VOCs were not estimated for the areas where the markers were below the limit of detection. Hodgson *et al.*, 1996, p. 301. This limitation makes Hodgson's results inapplicable to areas in office buildings or industrial facilities other than designated smoking areas. For example, these results are not applicable to rooms adjoining designated smoking areas, general work areas where smoking is allowed or nonsmoking areas.

3. ***Controlling Environmental Tobacco Smoke in Offices*, Ross, J.A.; Sterling, E.; Collett, Kjono, N.E.; HPAC [Heating/Piping/Air Conditioning], pp. 76-83, May 1996.**

Ross *et al.* assessed the effectiveness of air cleaners on ETS marker levels in a single office building: "[t]he objectives of the research were to assess the effectiveness of the air cleaners in providing acceptable indoor environmental conditions and to determine the impact of the air cleaning equipment on nonsmokers' exposure in the designated room." Ross *et al.*, 1996, p. 76.

Ross *et al.* found that at a smoking rate of 1.8 cigarettes per smoker per hour[9] and with conditions of adequate ventilation,[10] levels of ETS exposure markers were low in designated smoking areas and lower still in nonsmoking areas. These findings show that air cleaners can control ETS to low levels. Based on better markers of the ETS particle phase, *i.e.*, UVPM and FPM,[11] levels of ETS RSP were approximately 8 to 14 $\mu g/m^3$. Ross *et al.*, 1995, p. 81. Levels of the best marker of the ETS vapor phase, *i.e.*, 3-EP[12] were also low, ranging from about 1.0 to 1.2 $\mu g/m^3$.

In the designated nonsmoking area, levels of ETS markers were substantially less than in designated smoking areas. Based on UVPM and FPM, levels of ETS RSP are at most 8 $\mu g/m^3$ with the door to the area open and at or below the limit of detection with the door closed. 3-EP levels in the nonsmoking area with the door closed were at or below the limit of detection, 0.4 $\mu g/m^3$; with the door open, about 0.5 $\mu g/m^3$.

Ross *et al.* report concentration (or level) data without any corresponding duration data. Because duration data are absent, exposure cannot be quantified.[13] Although Ross *et al.* do not actually assess ETS exposures, the authors' selection of markers for assessing the concentration component of ETS exposures confirms the markers recommended by RJR and others.

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[9]This smoking rate is similar to rates reported by others. *See, e.g.*, RJR 1996 OSHA Post-Hearing Brief, Table 6, p. III - 41 (showing smoking rates ranging from 1.4 to 1.6 cigarettes per smoker per hour).

[10]Carbon dioxide was measured to assess whether ventilation was adequate. Levels ranged from 350 to 590 ppm, all less than 1000 ppm, the maximum permissible level recommended by ASHRAE Standard 62-1989. Ross *et al.*, 1996, pp. 81-82.

[11]*See* RJR, 1995 Cal/EPA Exposure Comments, p. 22.

[12]*See* RJR, 1995 Cal/EPA Exposure Comments, pp. 22-23.

[13]*See* RJR 1995 Cal/EPA Exposure Comments, p. 8.

a. Proper measurements of ETS require measurements of both the particle and vapor phases of ETS[14]

"Due to the dynamic and unpredictable nature of ETS, one can best characterize an accurate assessment of ETS concentrations in air by simultaneously monitoring selective particle-phase and vapor-phase tracers." Ross *et al.*, 1996, p. 78 (citation omitted).

b. FPM and UVPM are better markers than RSP for the ETS particle phase[15]

"[T]otal RSP concentrations provide an overestimation of ETS-related particulate matter. To provide a better estimate of ETS-related particulate concentrations, we also determined UVPM and FPM concentrations, which reflect concentrations of combustion-generated particulate matter, including ETS." Ross *et al.*, 1996, p. 78 (citation omitted).

c. 3-EP is a better marker than nicotine for the ETS vapor phase[16]

"[C]hamber experiments have questioned the validity of nicotine as an appropriate indicator due to unpredictable decay kinetics. Therefore, another vapor-phase tracer unique to tobacco smoke, 3-EP, was determined in addition to airborne nicotine." Ross *et al.*, 1996, p. 78 (citation omitted).

d. CO is a poor marker for the ETS vapor phase

"[T]he validity of CO as a tracer is limited since it is not unique to tobacco and field research has shown that ETS is a minor source of CO in office environments." Ross *et al.*, 1996, p. 78 (citation omitted).

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[14]*See* RJR 1995 Cal/EPA Exposure Comments, p. 19 ("[M]easurements of exposure to ETS require that markers be selected for both the vapor phase and the particulate phase of ETS.").

[15]*See* RJR 1995 Cal/EPA Exposure Comments, p. 22 ("[FPM] and UVPM are . . . better markers of ETS particulate phase than RSP.").

[16]*See* RJR 1995 Cal/EPA Exposure Comments, p. 22 ("[3-Ethenylpyridine] is the best available marker for the vapor phase of ETS.").

e. Most indoor RSP is not derived from ETS

Ross *et al.* confirm findings of other researchers that most indoor RSP comes from non-ETS sources.[17] Apportioning RSP based on UVPM and FPM levels, Ross *et al.* "estimate that between 30 and 40 percent of the [RSP] was related to ETS and other combustion byproducts." Ross *et al.*, 1996, p. 81.[18]

Summary of Results
(Concentrations visually estimated from Figures 3 and 4)

		Concentration	
Marker	Time of Day	Smoking	Nonsmoking
RSP ($\mu g/m^3$)	a.m.	23	13*
RSP ($\mu g/m^3$)	p.m.	38	29
UVPM ($\mu g/m^3$)	a.m.	10	2*
UVPM ($\mu g/m^3$)	p.m.	11	7
FPM ($\mu g/m^3$)	a.m.	8	2*
FPM ($\mu g/m^3$)	p.m.	14	8
Nicotine ($\mu g/m^3$)	a.m.	4	0.2*
Nicotine ($\mu g/m^3$)	p.m.	2.7	1
3-EP ($\mu g/m^3$)	a.m.	1	0.4*
3-EP ($\mu g/m^3$)	p.m.	1.2	0.5
CO (ppm)	a.m.	1.8	1.5
CO (ppm)	p.m.	1.9	1.8

* At or below detectable levels. (Ross *et al.*, 1996, p. 82.)

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[17]*See, e.g.* RJR, 1996 Cal/EPA Exposure Comments, p. 19; RJR, 1996 OSHA Post-Hearing Brief, p. II-25.

[18]This estimated range is fairly close to the 20 % figure that Baek *et al.*, discussed *infra*, pp. 19-20, report for the average contribution of ETS to RSP. Baek *et al.*, 1996, p. 488.

4. ***Estimation of ETS Contribution to RSP in Indoor Smoking Environments*, Baek, S.O., Kim, Y.S., Gee, I.; Indoor Air '96, Vol. I, pp. 483-488, 1996.**

Baek *et al.* attempted to collect data on the contribution of ETS to RSP in certain indoor environments in Korea. In this study, Baek *et al.* used area monitors to measure concentrations of five ETS markers for the particle and vapor phases: RSP, UVPM, FPM, Sol PM, and nicotine. Baek *et al.*, 1996, p. 484.[19] Baek *et al.* support the position taken by RJR and others that ETS differs from mainstream smoke ("MS"): "[R]elative quantities of many individual constituents present in ETS are considerably different from those found in mainstream smoke . . . ." Baek *et al.*, 1996, p. 483 (citation omitted).[20] Baek *et al.* demonstrate the benefits of using solanesol as a marker for the ETS particulate phase and the disadvantages of relying on airborne nicotine as a marker for the ETS vapor phase.[21]

a. Solanesol is the best marker for the ETS particulate phase[22]

Baek *et al.* recognize that, while FPM and UVPM are good markers for the ETS particulate phase, solanesol is the best available marker:

> From the viewpoint of the relative specificities of these two methods, the FPM method was known to be less prone to bias than the UVPM, and would be likely to overestimate the ETS contribution to RSP a lesser extent. On the other hand, the SolPM measurement was demonstrated by recent studies to be the best suited method to apportion total RSP into ETS and non-ETS contributions since solanesol

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[19]Use of area monitors -- as opposed to personal monitors -- was reasonable and appropriate for this study, since the objective was to investigate the contribution of ETS to RSP rather than to assess exposure.

[20]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 1-7.

[21]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 22-24 (identifying Sol PM as the best marker and UVPM and FPM as better markers than RSP).

[22]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 22-23; RJR 1996 OSHA Post-Hearing Brief, p. II-14.

> associated with airborne particles in indoor environments should be uniquely attributable to tobacco smoke.

Baek *et al.*, 1996, p. 486 (citations omitted). This study confirms that, in indoor sources where smoking occurs, about 50% of RSP originates from sources other than ETS.[23] Baek *et al.*, 1996, p. 488.

b. Nicotine is not the best marker for the ETS vapor phase[24]

Baek *et al.* recognize the limitations of using airborne nicotine as a marker for the ETS vapor phase:

> Although nicotine can be relatively easily analyzed, and it is present in both the vapor and particulate phases, problems associated with its use as a [sic] ETS marker have been recently demonstrated by several studies.

Baek *et al.*, 1996, p. 484 (citations omitted).

5. *Daily Exposure to Environmental Tobacco Smoke: Smokers vs. Nonsmokers in California*, Robinson, J.P., Switzer, P., Ott, W.; AJPH, Vol. 86, No. 9, pp. 1303-1305, 1996.

In this study, Robinson *et al.* attempt to assess ETS exposures in California using a 24-hour recall diary. As discussed in RJR's 1995 Cal/EPA Exposure Comments at pp. 11-13, questionnaires are the least reliable method of assessing exposures. Moreover, the 24-hour recall diary utilized by Robinson *et al.* appears to be materially identical to the method critiqued by Emmons *et al.*, discussed *supra*.

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[23]*See* RJR 1995 Cal/EPA Exposure Comments, p. 19 ("Ogden, measuring RSP and ETS-RSP with several different markers, found that total RSP measurements can overstate exposure to ETS-RSP by almost a factor of two.")

[24]*See also* RJR 1995 Cal/EPA Exposure Comments, pp. 14-17; RJR 1996 OSHA Post-Hearing Brief, p. II-23.

The questionnaire utilized by Robinson *et al.* obtained only limited information on duration and no information on concentration. Consequently, their questionnaire cannot provide information on exposure. The study relies on a single question to assess "exposure": "[w]ere you around anyone else who was smoking a cigarette, cigar, or pipe while you were (doing activity)?" Robinson *et al.*, 1996, p. 1304. This question does not address the "concentration" component of exposure. Maximum potential duration of exposure cannot be equated with actual ETS exposures. The data should be accorded little weight by Cal/EPA.

6. *Assessment of Non-Smokers' Exposure to Environmental Tobacco Smoke Using Personal-Exposure and Fixed-Location Monitoring*, Sterling, E.M., Collett, C.W., Ross, J.A.; Indoor Built Environ Vol. 5, pp. 112-125, 1996.

Sterling *et al.* conducted simultaneous personal exposure and fixed-location measurements in two office buildings. Exposure was based on markers for both the particulate and vapor phases of ETS. Sterling *et al.*, 1996, p. 113.[25] Sterling *et al.* measured as ETS markers RSP, UVPM, FPM, nicotine, 3-ethenylpyridine and solanesol. Exposure was assessed for the entire 8-hour workday. Sterling *et al.*, 1996, p. 113.

Sterling *et al.* provide extensive detail on the performance of the heating, ventilating and air conditioning ("HVAC") systems serving the buildings,[26] and on the procedures to quantify smoking activity. Documentation of this information (which allows assessment of study quality) is essential (but often lacking) in studies that attempt to assess exposure.

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[25]*See* RJR, 1995 Cal/EPA Exposure Comments, p. 19. ("Measurements of exposure to ETS require that markers be selected for both the vapor phase and the particulate phase of ETS.")

[26]*See* RJR 1995 Cal/EPA Exposure Comments, p. 25 (identifying ventilation rates as a criterion for study representativeness).

California EPA should not misinterpret Sterling *et al.*'s comparisons between personal and area monitors.[27] Sterling *et al.* recognized that area monitoring is inferior to personal monitoring by using personal monitors as the reference point for evaluating the quality of area monitoring results:[28]

> The overall results indicate that fixed-location monitoring provides a close approximation of an individual's exposure to ETS, as determined through personal monitoring. This finding suggests that the 'real world' data obtained by past researchers, primarily through fixed-location monitoring, is appropriate for estimating occupant exposure to ETS.

Sterling *et al.*, 1996, p. 112 (emphasis added).

The statistical test employed by Sterling *et al.* did not address the question of whether an area monitor could reliably indicate exposure measured by a personal monitor. Rather, this test addressed the question of whether the average concentration measured by two area monitors differed significantly from the average concentration measured by five to seven personal monitors. Sterling *et al.*, 1996, pp. 117, 121. Since both approaches are measuring only average concentrations in the building, it is not surprising that the data show that fixed area monitors and multiple personal monitors generally estimate similar average concentrations.

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[27]*See* Sterling *et al.*, 1996, pp. 117-18, 121 (discussing the "Comparison of Personal Exposure and Fixed-Location Data").

[28]*See* RJR, 1995 Cal/EPA Exposure Comments, p. 9. ("Personal monitoring is the only reliable means of determining an individual's actual exposure.")

7. ***Environmental Tobacco Smoke: Allegations of Scientific Misconduct*, Turner, S., Cyr, L., Gross, A.J.; Environment International, Vol. 22, No. 2 pp. 263-270, 1996.**

This editorial responds to accusations by James Repace and Alfred Lowrey regarding Simon Turner's 1992 study titled, "The Measurement of Environmental Tobacco Smoke in 585 Office Environments."[29] In this editorial, Turner, *et al.*, completely rebut the allegations raised by Repace and Lowrey. The authors regenerated the entire database used to write their 1992 paper and verified that the conclusions that they drew "are as valid now as they were then." Turner *et al.*, 1996, p. 267.

Turner *et al.* explain that Lowrey analyzed only 22 of the 585 data sets from Turner *et al.*'s original 1992 study. Lowrey's claimed criteria for selecting these data sets was that he chose the data sets where there was smoking and nonsmoking in the same building and where nicotine levels were below the limit of detection in nonsmoking areas. Turner *et al.*, 1996, p. 266. Although 130 data sets met Lowrey's criteria for evaluation, Lowrey chose the 22 data sets with the highest RSP values in the database, five of which did not even meet Lowrey's stated criteria. Turner *et al.*, 1996, p. 266. Additionally, Lowrey's analysis contains several gross miscalculations regarding smoking density in the data sets:

> Miscalculations: Smoking density in the smoking area should be 17.0 not 11.3 cig/ft².h (HBI page #684); smoking density should be 48.6, not 100 cig/ft².h (HBI page # 698); and smoking density should be 6.9 not 38 cig/ft².h (HBI page # 863).

Turner *et al.*,1996, p. 266.

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[29]This study was sponsored by Healthy Buildings International, Inc. ("HBI") and was provided to Cal/EPA by Philip Morris. *See* Turner, 1992 (provided to Cal/EPA in Philip Morris 1995 Cal/EPA Exposure Comments, Ref. 19). During OSHA's public hearings on its Proposed Rule on Indoor Air Quality, Gray Robertson, president of HBI was asked about the allegations of Repace and Lowrey. Robertson responded that these allegations were "clearly erroneous." Robertson, 1994 [OSHA Tr. p. 3340]. (References to the transcript from these hearings will be cited as "OSHA Tr. at ___.")

Turner *et al.* also point out that Lowrey's Table is missing one entire data set. Although the text states that 23 sets were selected, the table only reveals 22 data sets. "These errors call into question whether the table is a fair and representative comparison of smoking versus nonsmoking environments in our sample buildings." Turner *et al.*, 1996, p. 267.

In addition to disputing each allegation raised by Repace and Lowrey, Turner *et al.*, emphasize that Lowrey misrepresented Turner's data and misinterpreted their findings to draw unfounded and unwarranted conclusions. Additionally, Lowrey selected only the data that could be used to make his case and made major errors in assembling the data:

> Lowrey's poorly conceived criteria used to select these data sets, the cherry-picking from within this database, and the errors in the table Lowrey prepared indicate that the underlying analysis has no basis in fact.

Turner *et al.*, 1996, p. 267.

Thus, Turner *et al.*'s original conclusions that the model by Repace and Lowrey overestimate RSP levels four-fold and that, "under certain conditions of moderate smoking density and (most likely) good ventilation, indoor air quality was not significantly impaired by smoking activity," remain valid.

8. *Letters to the Editor*, Repace, J.L., Lowrey, A.H.; Environment International, Vol. 22, No. 2, pp. 268-270, 1996.

In reply to Turner's response to earlier accusations by Repace and Lowrey discussed above, Repace and Lowrey reiterate the positions taken by Lowrey in his 1994 paper but fail to address Turner's demonstration that Lowrey selected only the data that could be used to make his case. The authors provide no explanation for Lowrey's data selection methodology. Cal/EPA therefore should place no weight on their criticisms of Turner's study.

9. ***Exposure of the U.S. Population to Environmental Tobacco Smoke: The Third National Health and Nutrition Examination Survey, 1988 to 1991*, Pirkle, J.L., Flegal, K.M., Bernert, J.T., Brody, R.A., Maurer, K.R., JAMA, Vol. 275, No. 16, pp. 1233-1240, April 24, 1996.**

Pirkle *et al.* analyzed the data on serum cotinine levels and self-reported ETS exposure that were collected as part of Third National Health and Nutrition Survey ("NHANES III"). This paper confirms many of the analyses and results contained in RJR's 1996 Cal/EPA Exposure Comments, specifically the analyses of the NHANES III data by William J. Butler submitted with RJR's previous comments.[30] The findings in Butler's analyses and in Pirkle *et al.*, 1996 demonstrate many key points that Cal/EPA must understand concerning the NHANES III data on serum cotinine and self-reported ETS exposure. These key points include:

- U.S. EPA's adjustment for "background" levels of ETS exposure overestimated the number of lung cancer deaths statistically "attributable" to ETS exposure. Because Cal/EPA's 1997 Draft depends upon the U.S. EPA (1992) report for lung cancer deaths "attributable" to ETS exposure, the proportion of deaths in California is overestimated.

  - Pirkle *et al.*, 1996 (p. 1239) state that "[t]he NHANES III data suggest that there may be considerable exposure even within the comparison groups in studies of the effects of ETS. If so, these studies may underestimate the risk from ETS exposure." Pirkle *et al.*, 1996 appear to have overlooked the work by the U.S. EPA and others to adjust for this type of misclassification. Pirkle *et al.*, 1996 also appear not to have recognized that the NHANES III data can be used to estimate some of the quantities used in the U.S. EPA's adjustment algorithm.

  - The U.S. EPA developed the use of a "z-factor" to adjust their risk estimates for the "background" exposure to ETS among subjects with no self-reported ETS exposure. The z-factor is defined as the ratio of the mean nicotine exposure between nonsmokers married to a smoker and nonsmokers married to a nonsmoker.

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[30]RJR's 1995 Cal/EPA Exposure Comments referenced and attached two analyses by William J. Butler concerning the NHANES III data on ETS exposure. *See* RJR 1995 Cal/EPA Exposure Comments, pp. 29-31, 39-40 (citing Butler, 1995a and Butler, 1995b, respectively).

- Butler reported that the value of the z-factor calculated from NHANES III was approximately 4.8, almost three times higher than the value (1.75) used by U.S. EPA (Butler, 1995c, Table 3). Butler's value is similar to the ratio of the geometric means for those nonsmokers with and without household ETS exposure (5.6 = 0.700 ng/mL ÷ 0.124 ng/mL) reported in Pirkle *et al.*, 1996, Table 4. Thus, Pirkle *et al.*'s findings are consistent with Butler's and are inconsistent with the values used by EPA.

- Butler reported that the value of the z-factor he calculated from the NHANES III data decreases by more than half the number of lung cancer deaths statistically "attributed" by U.S. EPA to ETS.[31] A z-value computed from Pirkle *et al.*'s data further reduces this statistical estimate. Thus, this information from NHANES III shows that Pirkle *et al.*'s stated concern regarding the underestimation of the risk in epidemiologic studies of ETS is misplaced because the statistical methods used in the past have substantially over compensated for this type of misclassification. Pirkle *et al.*, 1996, p. 1239.

- Among adults, serum cotinine levels are associated with behavioral and demographic variables such as education, income, beer drinking, separation/divorce and region of the country. This statistical pattern demonstrates how the observed statistical associations of ETS with lung cancer and heart disease can result from uncontrolled confounding from recognized risk factors for these two diseases.

- Determining the contribution of diet to serum cotinine levels is complicated by the difficulty of measuring dietary nicotine and by ETS exposures encountered in different settings (*e.g.*, household, workplace, social, business, transport). It is not possible to place an upper limit on the amount of the variation in serum cotinine levels that could be explained by dietary factors either overall or in select subpopulations.

- The NHANES III data demonstrate that the correlation of serum cotinine levels with self-reported household and workplace ETS exposure may be overestimated due to confounding from unmeasured sources or characteristics of ETS exposure. These findings indicate that it is necessary to interpret the patterns of association of serum cotinine and self-reported ETS exposure in the context of potential confounding from other factors.

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[31]This process of statistically "attributing" deaths to exposure is purely a statistical exercise and does not reflect a causal relationship.

- The NHANES III data indicate that approximately 5.6% of adults in the U.S. population who report themselves to be never or former smokers are indeed current smokers who have used tobacco *recently*.[32]

10. ***Letter to the Editor Regarding: Estimating Exposure to Environmental Tobacco Smoke*, Coggins, C.R.E.; JAMA, Vol. 276, No. 8, p. 603, 1996.**

***Letter to the Editor Regarding: Estimating Exposure to Environmental Tobacco Smoke*, Domino, E.F.; JAMA, Vol. 276, No. 8, p. 603, 1996.**

***Letter to the Editor Regarding: Estimating Exposure to Environmental Tobacco Smoke*, Ogden, M.W.; JAMA, Vol. 276, No. 8, pp. 603-604, 1996.**

Three letters by Domino, Coggins, and Ogden critique the paper by Pirkle *et al.*, "Exposure of the U.S. Population to Environmental Tobacco Smoke," 275 J. Am. Med. Assoc. 1233 (1996). Domino criticizes Pirkle *et al.*'s conclusion that dietary sources of nicotine (which is metabolized to cotinine) make a negligible contribution to cotinine levels as compared to ETS. Domino recommends that the cotinine method of Pirkle *et al.* be used in a controlled experiment to measure the actual contribution of dietary sources of nicotine to levels of serum cotinine. Finally, Domino presents calculations indicating that the method could measure cotinine from the small amounts of nicotine that occur in some foods. Domino, 1996, p. 603.

Coggins responds to the statement by Pirkle *et al.* that "[f]urther research is needed to define better the degree of health risk associated with specific levels of cotinine." Pirkle *et al.*, 1996, p.

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[32]Cal/EPA's record contains substantial evidence of similar smoking status misclassification rates. *See, e.g.*, Oak Ridge National Laboratory ("ORNL") 1995, p. 31 (finding smoking status misclassification rates ranging from 3.0 to 6.0 percent); Ogden, 1995a, p. 4, Appendix A, pp. 21-22 (finding smoking status misclassification rates ranging from 2.8 to 4.1 percent); Butler 1996b, p. 1 (calculating genders-combined misclassification rates for recent users of tobacco from self-reported never- and former-users as: 5.6% (all subjects); 5.0% (married subjects); and 5.9% (currently employed subjects). *See also*, RJR 1996 Cal/EPA Lung Cancer Comments, pp. 26-27 (citing ORNL 1995; Ogden 1995a; and Butler 1996b for the above calculations).

1239. Coggins identifies animal studies which demonstrate that animals "had effectively no toxicologic changes" when cotinine levels were 165 mg/mL and particulate levels were 10 mg/m³. Coggins, 1996, p. 603. These results are particularly relevant because they represent cotinine levels several orders of magnitude greater than those that of Pirkle *et al.* reported for the U.S. population. Thus, Coggins's results strongly suggest that the cotinine levels reported by Pirkle *et al.* would be associated with "effectively no toxicologic changes" in humans.

Ogden observes that the cotinine results that Pirkle *et al.* report for nonsmokers living or working with smokers are similar to those found by earlier studies: "[a]s the data of Pirkle *et al.* show (Table 4), living with smokers results in at least a 2-fold increased exposure (as measured by cotinine) compared with working with smokers . . . ." Ogden, 1996, p. 603. Ogden also demonstrates that Pirkle *et al.*'s data regarding mean cotinine levels are consistent with previous results reported by Ogden. These conclusions further demonstrate that the EPA's adjustment for nonspousal exposure was too high and caused an inflated risk estimate:

> Pirkle *et al.* report mean cotinine levels for "exposed" (home ETS exposure only, cotinine level = 0.651 ng/mL) and "unexposed" (no home or work ETS exposure, cotinine level = 0.132 ng/mL) workers older than 16 years (Table 4). The ratio of these means (0.651/0.132 = 4.9) suggests that the EPA's adjustment for nonspousal exposure (which used a similarly derived ratio of 1.75) was too high, resulting in an inflated estimate of any risk from spousal exposure. the data of Pirkle *et al.* are consistent with data we reported recently (ratio = 4.8, based on cotinine).

Ogden, 1996, p. 603.

11. *Passive Smoking in a Displacement Ventilated Room*, Bjørn E., Nielsen, P.V.; Presented at the 7th International Conference on Indoor Air Quality and Climate in Nagoya, Japan, July 21-26, 1996.

Bjørn *et al.* identify two experimental objectives: (1) "to see if the displacement ventilation principle can protect a person from exposure to passive tobacco smoking" and (2) to determine "if

[ETS] stratification will always occur" in rooms because of temperature gradients, and if so, whether, "it will result in larger exposure to contaminants." Bjørn *et al.*, 1996, p. 887. It appears, however, that Bjørn *et al.* did not use tobacco smoke in their experiments. Bjørn *et al.* state that smoke was used to visualize air-flow patterns in the test room but fail to define the type of "smoke" used. Bjørn *et al.*, 1996, p. 889. Because smoke other than ETS is commonly used to visualize air-flow patterns, it is likely that these researchers did not use tobacco smoke.

In addition, not one ETS measurement is reported. Instead, Bjørn *et al.* measured di-nitrogenoxide (N_2O, nitrous oxide, commonly known as "laughing gas") as a surrogate for exhaled mainstream smoke. Bjørn *et al.*, 1996, p. 889. Moreover, Bjørn *et al.* did not attempt to account for sidestream smoke and, indeed, recognized this as a study limitation. Bjørn *et al.*, 1996, p. 892. Sidestream smoke is the main precursor of ETS.[33]

Despite the inability of Bjørn *et al.* to assess accurately ETS exposures, their experiment does identify one of the reasons why personal monitoring is superior to area monitoring for ETS exposure assessment: "[t]he convective boundary layer close to the human body modifies the concentration field locally, so that the inhaled concentration is different from the ambient concentration at the same height." Bjørn *et al.*, 1996, p. 892.[34]

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[33]*See* RJR, 1995 Cal/EPA Exposure Comments, pp. 1-3.

[34]*See also* RJR, 1995 Cal/EPA Exposure Comments, p. 9 (identifying personal monitoring as the only reliable means of assessing actual ETS exposure).

12. ***Personal Monitoring System for Measuring Environmental Tobacco Smoke Exposure*, Ogden, M.W., Heavner, D.L., Foster, T.L., Maiolo, K.C., Cash, S.L., Richardson, J.D., Martin, P., Simmons, P.S., Conrad, F.W., Nelson, P.R.; Environmental Technology, Vol. 17, pp. 239-250, 1996.**

This article was provided to Cal/EPA as part of RJR's Post-Hearing Comments to OSHA in 1995. Ogden, 1995b, App. D. Because it has now been published in a peer review journal, this article is being re-submitted to Cal/EPA in its published form.

13. ***Exposure to Environmental Tobacco Smoke in Sixteen Cities in the United States as Determined by Personal Breathing Zone Air Sampling*, Jenkins, R.A., Palausky, A., Counts, R.W., Bayne, C.K., Dindal, A.B., Guerin, M.R.; Journal of Exposure Analysis and Environmental Epidemiology, Vol. 6, No. 4, pp. 473-502, 1996.**

The preliminary results for this study were provided to Cal/EPA as part of RJR's October 16, 1995 comments. Because it has now been published in a peer review journal, this article is being re-submitted to Cal/EPA in its published form.

14. ***Environmental Tobacco Smoke (ETS): A Market Cigarette Study*, Martin, P., Heavner, D.L., Nelson, P.R., Maiolo, K.C., Risner, C.H., Simmons, P.S., Morgan, W.T., Ogden, M.W.; Environment International, Vol. 23, No. 1, pp. 75-90, 1997**

This paper provides an in-depth analysis of the ETS yields for the top 50 selling cigarettes. In addition to providing useful information regarding overall yields for the selected analytes, this study also demonstrates the futility of relying on nicotine-RSP ratios. The authors demonstrated that there is a poor relationship between ETS nicotine and ETS RSP.

B. Chapter 3-4: Developmental Toxicity

In Chapter 3 (Developmental Toxicity I: Perinatal Manifestations) and Chapter 4 (Developmental Toxicity II: Postnatal Manifestations) of the 1997 Draft, Cal/EPA reviews the scientific literature regarding ETS and developmental effects. In Appendix A to these comments,

Dr. James Swauger reviews and analyzes the scientific evidence on ETS and developmental effects and concludes that the available data are inadequate to support Cal/EPA's conclusions. Dr. Swauger's comments focus on the new information identified by Cal/EPA in the 1997 Draft. In addition, Dr. Swauger raises new issues not previously considered by Cal/EPA.

In Chapter 3, Cal/EPA concludes that ETS exposure is causally associated with decreased fetal birthweight. Appendix A of these comments reviews in detail the six recent epidemiologic studies cited by Cal/EPA to support this conclusion. These six epidemiologic studies were not considered by Cal/EPA in the External Review Draft Chapter on Developmental Effects. Swauger's comments raise the following issues:

- The published literature on ETS and fetal birthweight is weak and inconsistent
- The epidemiologic studies do not consistently control for important confounders in studies of fetal birthweight. These confounders include: gestational age, race, birth order, education level, maternal age, paternal smoking behavior, maternal weight, weight gain during pregnancy, maternal height, dietary patterns, smoking status, alcohol use, caffeine intake, social status, fetal sex, and gravidity.
- The epidemiologic studies do not sufficiently control for smoking status misclassification bias
- Prior knowledge of birth outcome can result in recall bias due to selective reporting of smoking behavior and ETS exposure by new mothers.
- None of the epidemiologic studies attempt to quantitatively measure ETS exposures
- The epidemiologic studies of ETS and fetal birthweight show an absence of a dose-response relationship
- Animal models have provided limited and inconsistent results

(Swauger 1997, Appendix A)

In Chapter 4, Cal/EPA concludes that ETS is causally associated with sudden infant death syndrome ("SIDS"). Dr. Swauger's comments review in detail the five recent epidemiologic studies

cited by Cal/EPA to support this conclusion. These five epidemiologic studies were not considered by Cal/EPA in the External Review Draft Chapter on Developmental Effects. Dr. Swauger's comments raise the following issues:

- The epidemiologic studies of ETS and SIDS are weak and inconsistent
- The epidemiologic studies do not consistently control for important confounders in epidemiologic studies of SIDS. These confounders include: region, time of day, marital status of mother, maternal smoking, season, maternal age, parity, ethnic group, fetal birthweight, fetal age, breast feeding, social status, sleep position, and bed sharing.
- The epidemiologic studies do not adequately control for misclassification of smoking status.
- Two epidemiologic studies cited by Cal/EPA (Milerad *et al.*, 1994 and Haglund *et al.*, 1995) did not distinguish between active maternal smoking during pregnancy and postnatal ETS exposure. Therefore, the methodology of the studies does not allow an appropriate examination of postnatal ETS exposure and SIDS.
- None of the epidemiologic studies attempt to quantitatively measure ETS exposures.
- The epidemiologic studies of ETS and SIDS do not show a dose-response relationship
- Three epidemiologic studies cited by Cal/EPA (Mitchell *et al.*, 1995, Klonoff-Cohen *et al.*, 1995, and Blair *et al.*, 1996) collected information regarding smoking behavior (i.e., exposure) following the death of the infant, in some cases years later. Prior knowledge of the outcome may have resulted selective recall bias in reporting smoking behavior and ETS exposures.

(Swauger, 1997, Appendix A)

Cal/EPA must consider, analyze, and address these issues and their impact on the conclusions concerning ETS and developmental effects (fetal birthweight and SIDS) in Chapters 3-4 of the 1997 Draft.

C. Chapter 6: Respiratory Health Effects

Chapter 6 (Respiratory Health Effects) of Cal/EPA's 1997 Draft purports to review "a substantial body of literature" that addresses acute and reversible irritative effects and ETS. (Cal/EPA 1997 Draft, Section 6.1.4, pp. 6-27 to 6-32). In Appendix B to these comments, Dr. James C. Walker illustrates major methodologic shortcomings in Cal/EPA's review of the available data concerning ETS and sensory irritation and annoyance. Dr. Walker's comments point out the following deficiencies that must be addressed by Cal/EPA:

- Cal/EPA should use operational definitions to lessen the ambiguity of using words like "irritation" that have multiple meanings.
- Cal/EPA should pay close attention to real-world ETS concentrations and exposures, and analyze whether the hypothesized effects asserted by Cal/EPA have been shown to occur in environmentally realistic ETS conditions.
- Cal/EPA presents a great deal of information irrelevant to a scientific discussion on the issue of perceived odor or irritation and ETS.
- Cal/EPA should delete phrases like "mucous membrane symptom" that add confusion and are not defined.
- Cal/EPA should eliminate or adequately characterize the results of the experimental studies that have not been replicated (e.g., Ahlstrom *et al.*, 1987).
- Cal/EPA must include recently published studies that are directly relevant to sensory impact and ETS, which were conducted in representative current indoor air environments.
- Cal/EPA relies excessively on second or third-order reviews and opinions that are of dubious quality. Cal/EPA should focus on the peer-reviewed literature and original research data.

(Walker, 1997, Appendix B).

Cal/EPA must consider, analyze, and address these issues and their impact on the conclusions concerning ETS and Sensory Irritation and Annoyance in Chapter 6 (Section 6.1.4) of

the 1997 Draft. A proper analysis of these issues and new information submitted by RJR will invalidate Cal/EPA's conclusion that nonsmokers can be protected against sensory irritation and odor annoyance only through a prohibition of smoking or extreme engineering measures.

D. Chapter 7: Carcinogenic Effects

On April 1, 1996, R. J. Reynolds Tobacco Company ("RJR") submitted comments ("RJR 1996 Cal/EPA Lung Cancer Comments") on the California Environmental Protection Agency's ("Cal/EPA") January 1996 External Review Draft, titled "Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer" ("Lung Cancer External Review Draft"). RJR submits new information that Cal/EPA must consider as it revises the lung cancer chapter of the 1997 Draft.

As RJR explained in its 1996 Cal/EPA Lung Cancer Comments, Cal/EPA must: 1) consider all relevant studies of ETS and lung cancer; 2) identify the studies that comprise the best available evidence; and 3) base any conclusions on the best available evidence. As demonstrated in RJR's 1996 Cal/EPA Lung Cancer Comments, the Lung Cancer External Review Draft did not fulfill Cal/EPA's obligations. Moreover, new information has become available since the Lung Cancer External Review Draft was released for comment. As a result, the analysis contained in Chapter 7 of the 1997 Draft is incomplete and uninformed by important new findings. Cal/EPA must consider this new information.

The supplemental evidence presented here consists of 17 new publications and a discussion of the implications of the information presented in the publications.

1. ***Environmental tobacco smoke and lung cancer mortality in the American Cancer Society's Cancer Prevention Study II,*** **V. M. Cardenas, M. J. Thun, H. Austin, C. A. Lally, W. S. Clark, R. S. Greenberg and C. W. Heath Jr.; Cancer Causes Control (1997); 8:57-64**

Environmental Tobacco Smoke and Lung Cancer Mortality In the American Cancer Society's Cancer Prevention Study II, **V. M. Cardenas; A dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy (1995)**

Both the 1997 publication and 1995 dissertation examine ETS exposure surrogates and lung cancer deaths in the American Cancer Society cohort study, Cancer Prevention Study-II (CPS-II). Both documents report that the risk of lung cancer attributed to ETS exposure is not statistically significant. These results are consistent with other evidence submitted by R.J. Reynolds on this issue and are inconsistent with the conclusions reached by Cal/EPA. Most relative risk estimates reported in the 1997 paper are slightly higher than corresponding estimates in the 1995 dissertation.

The two publications differ substantially in the number of subjects considered for the "spousal" exposure results because the authors made an important change in subject-exclusion criteria. In the 1995 dissertation, approximately 20,000 subjects (including 16 cases) who otherwise met the inclusion criteria but had a blank entry for spousal smoking are assigned to the spousally-unexposed category. The 1997 paper does not include subjects with blank entries in its analyses.

The two publications differ only slightly in the number of subjects considered for the "self-reported" exposure results. Contrary to the significant change in exclusion criteria that affected the "spousal" results, the criteria were not changed for the "self-reported" results: subjects with blank exposure values are assigned to the unexposed category in both publications for the "self-reported" analyses. The authors justify this (unchanged) approach by indicating that CPS-II exposure prevalence rates matched most closely to the 1988 NHIS prevalence rates when this was done. They

give no explanation as to why this reasoning does not apply equally well to the exclusion rules for spousal exposure results.

The spousal exposure results in Table 3 of the 1997 paper show generally higher RR's than given in the 1995 dissertation but, as noted above, are based on fewer subjects. The RR's in the age-adjusted column are acknowledged by the authors to be incorrect (an erratum is forth-coming). The self-reported dose-level results are shown in Table 4. The reported counts match closely with those in the 1995 dissertation but the dose-level RR's are usually higher. There is no apparent explanation for the discrepancies in these RR's.

In their 1997 discussion, the authors appear to have abandoned the long-standing statistical principle that non-significant results do not indicate an association; instead they choose to view the U.S. EPA's estimate of a (slightly) elevated risk from ETS (based on pre-1992 data) as the null hypothesis that new studies must significantly disprove. Cal/EPA's 1997 draft uses this same faulty approach in evaluating the impact of additional ETS and lung cancer studies.

Cardenas' 1995 dissertation demonstrates the importance of many potential confounders of the ETS epidemiologic studies. However, the 1997 paper does not include these analyses.[35] Potential confounders that are considered and incorporated in the analyses in the 1995 dissertation are: age, gender, race, education, carotenoid intake, fat intake, asbestos exposure, and history of chronic lung disease. Many of these potential confounders are statistically significant or have relative risks larger than the RR's estimated for ETS in this data set. Therefore, even minor uncontrolled differences in the occurrence of these confounders between exposed and unexposed

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[35]A failure to include a full description of the magnitude of the effect of confounders or alternative risk factors is common in the ETS and lung cancer epidemiologic publications. Cal/EPA should acknowledge that this absence reduces the ability to draw conclusions from such studies.

subjects can greatly affect the ETS relative risk estimate. The RR's provided in the 1995 dissertation for these potential confounders are shown in the table below (statistically significant RR's are italicized).

Potential confounder	Combined Genders Cox adjusted models RR (95% CI)	Potential confounder	Combined Genders Cox adjusted models RR (95% CI)
Age (vs. <50)		Lung Disease (vs. None)	
50-54	1.8 (1.0-3.0)	Any	1.0 (0.7-1.5)
55-59	*2.9 (1.8-4.7)*	Tuberculosis	1.1 (0.4-2.6)
60-64	*4.4 (2.7-7.0)*	Emphysema	1.8 (0.8-4.2)
65-69	*6.1 (3.8-9.9)*	Asthma	1.7 (0.9-3.6)
70-74	*8.2 (5.0-13.3)*	Bronchitis	1.2 (0.7-2.1)
75-79	*14.4 (8.8-23.9)*		
80-84	*14.6 (8.1-26.6)*		
85+	*22.4 (11.6-43.6)*		
Carotenoid Foods (vs. Never)		Fat (vs. Lowest quintile)	
1-2/week	*0.3 (0.1-0.8)*	2nd	1.2 (0.7-1.5)
3/week	*0.3 (0.1-0.7)*	3rd	1.3 (0.9-1.8)
>3/week	*0.3 (0.1-0.7)*	4th	0.9 (0.6-1.3)
		Most	*1.7 (1.2-2.3)*
Race (non-white vs. white)	*1.5 (1.1-2.2)*	Gender (Male vs. Female)	1.3 (1.0-1.6)
Schooling (<12 years vs. 12+ years)	1.2 (0.9-1.5)	Lung Carcinogens @ Work (Ever vs. Never)	0.9 (0.6-1.3)
Asbestos (Ever vs. Never)	*1.8 (1.1-3.2)*	Radiation (Ever vs. Never)	1.5 (0.7-3.5)

The analyses of the nonsmoker sub-sample in both documents are age-gender adjusted to match the overall CPS-II cohort (but not the US population). Because the CPS-II cohort is not representative of the U.S. population as a whole,[36] Cardenas' adjustment of the data for these demographic variates provides results that may not be appropriate for the U.S. population.

[36]Cardenas' 1995 dissertation provides a detailed description of the demographics of the CPS-II cohort (this detailed description is absent from the 1997 paper). Among the demographics that Cardenas describes that are not representative of the U.S. population are differences in race, gender, age, employment, education, marital status, and lifestyle. His observations confirm that the CPS-II data set is not representative of the U.S. population. (Cardenas, 1995, pp. 54-60).

2. *Environmental Tobacco Smoke and Lung Cancer: An Evaluation of the Risk*, Idle, J., Benitez, J., Krokan, H.E., Lohman, P.H.M., Roberfroid, M., Springall, A.; Report of A European Working Group, April 1996.

This report ("Idle *et al.*"), prepared by a panel of European scientists, evaluates ETS and lung cancer. As discussed below, Idle *et al.*'s analysis of issues pertaining to the epidemiologic studies provides additional support for many of the points demonstrated in the RJR 1996 Cal/EPA Lung Cancer Comments.

a. The Idle *et al.* analysis is inconsistent with U.S. EPA's conclusions[37]

Idle *et al.* analyze 48 epidemiologic studies related to ETS and conclude that ETS is not a primary lung carcinogen. Idle *et al.* 1996, p. 91. The authors conclude further that "these studies do not support an elevated risk of lung cancer due to ETS exposure in the workplace" and that "the available epidemiological studies do not support an elevated lung cancer risk due to exposure to environmental tobacco smoke." Idle *et al.* 1996, pp. 41, 59.

b. U.S. EPA failed to consider numerous sources of bias and confounding in spousal epidemiologic studies[38]

As explained by Idle *et al.*, the exposure surrogate utilized in spousal studies introduces bias and confounding:

> Use of the spouse's smoking habit to divide subjects into those exposed and those not exposed to ETS . . . assumes . . . they have lifestyles, in as far as risk factors for lung cancer are concerned, which are otherwise identical.

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[37]The authors reported a relative risk ("RR") consistent with the RRs cited by RJR: "If the RR's from these workplace studies are combined . . . a pooled estimate of 1.04 (95% CL: 0.95-1.14) is obtained. Similarly, if the US results . . . are combined a pooled estimate of 1.02 (95% CL: 0.93-1.13) is obtained." Idle *et al.* 1996, p. 40. *See* RJR 1996 Cal/EPA Lung Cancer Comments, p. 47.

[38]*See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 17-22.

Idle *et al.* 1996, p. 48. Idle *et al.* demonstrate that merely adjusting for smoking status misclassification bias alone in the spousal studies reduces the reported relative risk essentially to 1.0:

> Using Lee's method . . . an overall smoker misclassification bias estimate for the US studies of 1.14 is obtained. Such a smoker misclassification bias reduces the RR for US [spousal] studies to 1.01 (95% CL: 0.90-1.13).

Idle *et al.* 1996, p. 46.[39]

c. A meta-analysis is inappropriate when the studies are heterogeneous[40]

Idle *et al.* stress that the studies used in a meta-analysis must be homogeneous:

> Estimation meta-analysis reasons that if the results from the studies are numerically combined, the composite relative risk estimate will better reflect the "true" relative risk. This is only true if the studies are unbiased as a group and are homogeneous.

Idle *et al.* 1996, p. 40.

d. The technique used by Fontham to detect misclassification of smoking status introduces a bias that elevates the observed risk estimate[41]

With respect to smoking status misclassification, Idle *et al.* conclude that Fontham's methodology actually increased the potential bias from smoking status misclassification:

> It is worth noting that the special methods employed by Fontham *et al.* (1994) to eliminate misclassified smokers involved testing subjects' urine for cotinine and eliminating those whose cotinine level suggested that they were a smoker. Perversely, this special care to remove current smokers may have increased the bias. This arises because a lower proportion of the cases (53.5%) than the controls (83.3%) were tested and thus more of the misclassified controls than the misclassified cases were detected and removed.

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[39]*See* RJR 1996 Cal/EPA Lung Cancer Comments, p. 28.

[40]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 43-46; *see also* discussion below regarding Moolgavkar *et al.*, 1996.

[41]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 56-60.

Idle *et al.* 1996, p.46.

e. The inability to control adequately for bias and confounding is a major weakness of epidemiologic studies[42]

Idle *et al.* discuss the limitations of epidemiologic data, including the inability to control for biases and confounders:

> An epidemiological study is, in general, an unsatisfactory, and potentially misleading, method of examining the true association between a disease and a risk factor since it is very susceptible to a wide variety of biases.

Idle *et al.* 1996, p. 27.

> Epidemiological studies are observational studies and, like market research surveys and opinion polls, lack the experimental design control that is normally associated with scientific experiments. . . . This means that they are very vulnerable to two problems. First, they tend to be imprecise because of their frequent reliance on questionnaire information and human memory. This places a lower boundary on the size of associations they can detect. Second, they are susceptible to the presence of biases. If these biases are of a similar order of magnitude to the possible size of the effect sought, this can lead to erroneous conclusions.

Idle *et al.* 1996, p. 58.

f. Diet is a critical confounding factor that must be examined in studies of ETS and lung cancer[43]

"[I]t is also important to emphasize that various food products often contain most, if not all, of the chemicals detected in ETS, and that the amounts of these contaminants in the daily diet, in most cases, exceeds by far the amount that might be taken up by inhaling ETS in the indoor air." Idle *et al.* 1996, p. 61.

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[42]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 5-12.

[43]*See* Steichen 1995a; *see also* Butler 1996a, pp. 9-12; RJR 1996 Cal/EPA Lung Cancer Comments, pp. 17-21.

g. A trend test is not a dose response test[44]

"[A] trend test is not a dose-response test." Idle *et al.* 1996, p. 53.

h. Publication bias is evident in the ETS literature[45]

"It is likely that the majority of studies did carry out such a [trend] test but failed to report it when nonsignificant." Idle *et al.* 1996, p. 53.

3. *An Alternative Explanation for the Apparent Elevated Relative Mortality and Morbidity Risks Associated with Exposure to Environmental Tobacco Smoke*, Sterling, T.D., Glicksman, A., Perry, H., Sterling, D.A., Rosenbaum, W.L., Weinkam, J.J.; Journal of Clinical Epidemiology, Vol. 49, pp. 803-808, 1996.

Sterling *et al.* address socioeconomic status and paraoccupational exposure[46] as confounders in the studies on ETS, mortality and morbidity:

> Insofar as industrial and other blue collar workers are now likely to bring home toxic materials on their person, and also are more likely to smoke than those in other occupations, members of a household are much more likely to be subject to a paraoccupational exposure and belong to lower socioeconomic strata if the household contains a smoker than if the household does not contain a smoker.

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[44]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 37-40.

[45]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 29-34.

[46]Sterling *et al.* define paraoccupational exposure as, "an exposure to a substance outside the occupational setting in which workers are exposed to that substance. It usually is an exposure of a worker's family to materials brought home on the hair, skin or clothing of the worker." Sterling *et al.* 1996, p. 803.

Sterling *et al.* 1996, p. 803.[47] Sterling *et al.* conclude that any observed increase in mortality or morbidity that is associated with household ETS exposures, "may be partly or entirely due to differences in paraoccupational exposure or socioeconomic strata." *Id.*[48]

Sterling *et al.* find further that none of the ETS epidemiologic studies have controlled for paraoccupational exposures: "[Our review reveals] not a single instance in which published risk estimates were adjusted for paraoccupational confounding, although a few reports have considered either husband's occupation or socioeconomic status as confounders." *Id.* at 806.

Because adjustment for confounders is critical when the epidemiologic studies in question demonstrate a weak association, as is the case with the studies of ETS and lung cancer, Cal/EPA must reevaluate the failure of the published studies to account for paraoccupational exposures as a potential confounder.[49]

4. ***Environmental Tobacco Smoke and Lung Cancer: A Reappraisal*, Nilsson, R.; Ecotoxicology and Environmental Safety, Vol. 34, pp. 2-7, 1996.**

This paper is a recent review of several issues related to ETS and lung cancer. Nilsson's conclusion is that "most of the increase reported in the epidemiological studies linking ETS with lung cancer can be adequately explained by misclassification of smoking status, unequal inclusion among cases and controls of groups of disease-prone individuals from low socioeconomic

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[47]*See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 18-22.

[48]Sterling *et al.* note also that, "findings of the extensiveness of paraoccupational exposures are strong enough to have motivated the U.S. Congress to pass a special bill, the Workers' Family Protection Act, Public Law No. 102-522 [30], which instructs the Director of the National Institute for Occupational Safety and Health to investigate the risk to household members from carcinogens brought home on the persons of industrial workers." *Id.* at 805.

[49]*See* discussion regarding "strength of association" in RJR's 1996 Cal/EPA Lung Cancer Comments, pp. 5-12; *see also* Butler 1996a, pp. 29-37.

background, as well as by certain confounding factors related to life-style and diet." Nilsson 1996 p. 14.

Nilsson makes the following points, among others, that corroborate the positions taken in RJR's 1996 Cal/EPA Lung Cancer Comments.

a. Histological inconsistencies

Nilsson finds that there are histologic inconsistencies among the tumors reported in the epidemiologic studies that undermine the confidence that can be placed in the epidemiologic studies reporting increased risks:

> All established human lung carcinogens . . . seem preferentially to induce tumors of the Kreyberg type 1. It is, therefore, biologically highly implausible that . . . ETS should induce mainly adenocarcinomas.

Nilsson 1996, p. 5.

b. Confounding[50]

Nilsson points out that in the Fontham *et al.* 1994 study, the method used for selecting controls, *i.e.* random digit dialing, made it much less likely that the control group would include persons of low socioeconomic status. The method utilized by Fontham *et al.* for selecting cases, however, did not have this effect because persons with low socioeconomic status utilized the hospitals where Fontham *et al.* recruited their ETS cases. Nilsson 1996, p. 10. This in fact occurred in the Fontham data. Nilsson demonstrates that Fontham's cases had lower education and lower incomes than the controls:

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[50]*See* RJR 1996 Cal/EPA Lung Cancer Comments; Steichen 1995a; *see also* Butler 1996a, pp. 9-12.

> [T]here were about 50% more cases than controls with household incomes below $8,000. Further, around 60% more cases than controls did not even have high school education.

Nilsson 1996, p. 10.[51]

Nilsson also addresses the potential confounding from dietary factors. In particular, Nilsson cites the significant role that dietary fat played in the Alavanja study:

> [T]he relative risk among nonsmoking women observed with increased saturated fat consumption was more than 6-fold greater for the highest quintile than for the lowest quintile. The effect of fat intake was more pronounced for adenocarcinoma than for other cell types. For adenocarcinoma there was an 11-fold elevation in risk in the highest versus lowest quintiles of saturated fat consumption. . . . [T]he professed effect of ETS is barely visible in comparison with the effect of saturated fat.

Nilsson 1996, p.10.

c. Misclassification[52]

Nilsson demonstrates that recall bias results when information on smoking status is derived solely on the basis of interviews:

> In the epidemiological studies on ETS, with a few notable exceptions, information on smoking status have solely been derived by interview. The unreliability of information on smoking status, especially for previous smoking habits, obtained in this manner has been well documented.

Nilsson 1996, p.6.

Nilsson also discusses the potential bias introduced by using surrogate sources as the only source of information regarding the smoking status of a deceased subject:

> If, *e.g.*, a deceased mother was for many years a nonsmoker before her death, common sense dictates that it is far more likely that the children will describe her as a lifelong nonsmoker than as an ex-smoker.

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[51]RJR 1996 Cal/EPA Lung Cancer Comments; Steichen 1995c.

[52]*See* Butler 1995, p. 6.

Nilsson 1996, p.7.[53]

Nilsson also addresses the inability of cotinine measurements to assess smoking status misclassification rates:

> There is no doubt that cotinine determination in the majority of cases adequately discriminates between active smokers and nonsmokers. However, many epidemiologists, NRC, U.S. E.P.A., and OSHA have all made the cardinal mistake to assume that determination of cotinine would be sufficiently accurate for use in this context , i.e. to assure that misclassification in the range of 5-10% does not occur.

Nilsson 1996, p. 7.[54]

5. *Environmental Tobacco Smoke*, Law, M.R., Hackshaw, A.K.; British Medical Bulletin, Vol. 52, No. 1, pp. 22-34, 1996.

Law and Hackshaw superficially review the ETS literature in what appears to be a search for evidence to support a claim of detrimental effects from ETS exposure. Many of the conclusions that Law and Hackshaw reach are based on dated information. Law and Hackshaw provide no original data.

For lung cancer, Law and Hackshaw base a projected ETS relative risk of 1.2 on Wald's 1984 work concerning active-smoking risk and differences between ETS exposure and active smoking. Law and Hackshaw then assume, based on ETS studies published through early 1994, and not on any original data of their own, that worldwide ETS studies can be combined into a single meta-analyzed estimate of 1.24. This estimate has little value for Cal/EPA because it is not based on U.S. studies and is contradicted by the meta-analyses in the record that are restricted to U.S. data. The meta-analysis of U.S. spousal studies provided to Cal/EPA by RJR, for example, yields an

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[53]*See* Ogden 1995a, p. 4, Appendix A, pp. 21-22.

[54]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 47-48.

uncorrected OR of 1.08 (95% CI: 0.95-1.22) and an OR of 1.0 (95% CI: 0.88-1.14) (adjusted for misclassification of regular smokers as never smokers).[55]

Law and Hackshaw also conjecture self-servingly regarding confounding and bias. They dismiss dietary confounding by citing older epidemiology studies that supposedly "adjusted" for it and found no change in risk. This approach does not address confounding introduced by factors not studied or not completely controlled for in the epidemiologic studies. For example, they fail to address socioeconomic status,[56] smoking status misclassification,[57] or any of several other biases and confounders introduced by the use of spousal exposure studies.[58] Cal/EPA should accord Law and Hackshaw's analysis no weight in its revised assessment.

6. ***Whose data are they anyway*? Delamothe, T.; British Medical Journal, Vol. 312, pp. 1261-1262, May 18, 1996.**

Delamothe addresses the importance of sharing data and making it available to others for re-analysis. RJR submits this article to Cal/EPA as further evidence that Cal/EPA should obtain and make available to all interested parties the raw data from the study by Fontham *et al.*, titled, "Environmental Tobacco Smoke and Lung Cancer in Nonsmoking Women."[59] Because Cal/EPA

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[55]Although RJR has demonstrated that it is inappropriate to apply meta-analysis to the entire body of ETS lung cancer data, the analysis is presented here because this technique has been suggested as a way that Cal/EPA should analyze the data on ETS and lung cancer. *See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 46-47 (several assessments of the ETS data using meta-analysis were submitted to the record, all of which had RRs of approximately 1.0).

[56]*See* RJR 1996 Cal/EPA Lung Cancer Comments, p. 17; Steichen 1995c.

[57]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 23-28; Ogden 1995a, p. 4, Appendix A, pp. 21-22.

[58]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 17-23.

[59]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 66-68.

places such heavy reliance on this study, many scientific reviewers of the study have called for the Fontham data to be made available for reanalysis:

> [Independent statistical and epidemiologic analyses] are needed to verify and examine in greater depth the findings reported by Fontham. . . . [t]he amount and detail of information from an epidemiologic study that is published in a peer reviewed journal may not be a sufficient summary of all the data from that study that is relevant to the development of public policy and regulatory decisions.

Butler 1995, p. 12; *see also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 66-68.

Calls for the production of the Fontham data are not limited to persons critical of the Fontham study. Steven Bayard of U.S. EPA agrees that the Fontham data should be subjected to reanalysis. Using Butler's detailed analysis of Brownson (1992) as an example, Bayard testified in the OSHA public hearings on its Proposed Rule on Indoor Air Quality that the reference group used by Fontham for the analysis of the workplace data -- which includes women who were not employed outside the home -- is inappropriate, and that the analysis should be performed using only women employed outside the home.[60] Bayard 1994, 1995; OSHA Tr. at 14722-23, Slide 43 (Bayard).[61]

The requests for the Fontham raw data are consistent with established scientific principles. Epidemiologic societies encourage sharing data so that research findings can be replicated. International Epidemiology Association 1990; Society for Epidemiologic Research 1989. The

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[60]A recent letter to the editor from Fontham *et al.* purports to provide such an analysis but is not adjusted for the effects of the interaction documented by Butler. Butler 1996c; RJR 1996 Cal/EPA Lung Cancer Comments, pp. 66-67.

[61]Bayard expressed his preference for sharing data and "expects that everyone would want to share data on health effects." OSHA Tr. at 14962-14963 (Bayard). *See also* OSHA Tr. at 6536 (Starr) (providing raw data in risk assessments for public purposes is laudatory and every attempt should be made [to provide the data]); Butler 1996c, p. 23 (citing Glantz 1992, p. 8 ("except when ... a paper includes the raw data, a reader cannot tell whether the data in fact support the author's conclusions or not").

largest epidemiologic society in the United States recommends that "when epidemiologic data are relevant to the governmental decision making process including public policy and regulatory decisions, investigators should share the data tapes and records as promptly and expeditiously as possible. . . ." Society for Epidemiologic Research 1990. The National Research Council also recommends that "data relevant to public policy be shared as quickly and widely as possible" and that "investigators should share their data by the time of publication of initial major results of analyses of the data except in compelling circumstances." NRC 1985, p. 26.

7. *A Critical Review of the Evidence on Particulate Air Pollution and Mortality*, Moolgavkar, S.H., Luebeck, E.G.; Epidemiology, Vol. 7, pp. 420-428, 1996.

This recent and highly instructive paper reviews epidemiologic data on particulate air pollution (*i.e.* outdoor air) and mortality.

a. Meta-analysis is inappropriate when the studies are heterogeneous[62]

> [W]e believe that the various epidemiologic studies of particulate air pollution and mortality are sufficiently different in their measures of exposure, control of confounders, and methods of analysis that a single overall estimate of risk derived from the totality of studies cannot be defended.

Moolgavkar and Luebeck 1996, p. 420. Moolgavkar and Luebeck conclude further that heterogeneity of studies is actually the key problem limiting the utility of meta-analysis:

> The main problem faced by the meta-analyst can be summed up in a single word: heterogeneity. Whether it is heterogeneity in the probability of publication (publication bias), in the quality of studies, in the measures of exposure or response used, or the control of confounders, the analyst must be prepared to deal with it.

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[62]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 43-46; *see also* discussion, *supra*, pp. 37-40, regarding Idle *et al.*

Moolgavkar and Luebeck 1996, p. 426. These concerns are directly applicable to Cal/EPA's development of a valid ETS risk assessment. In RJR's 1996 Cal/EPA Lung Cancer Comments, RJR explained that, "methods of meta-analysis are intended only for highly similar studies, a condition not present in the ETS epidemiology studies." RJR 1996 Cal/EPA Lung Cancer Comments, pp. 44-45.

b. Weak associations are especially prone to confounding[63]

> [C]onfounding is by far the most important issue in epidemiological studies, such as those of air pollution, in which relative risks are small. Inadequate control of confounding could very easily lead to spurious relative risks of the magnitude (typically less than 1.2) reported in studies of air pollution.

Moolgavkar and Luebeck 1996, p. 421.

8. *Response to Science article, Epidemiology faces its limits*, Wynder, E.L., American Journal of Epidemiology, Vol. 8, pp. 747-749, 1996.
AND
***Epidemiology, risk assessment, and public policy: Restoring epistemic warrants*, Gori, G.B.; Risk Analysis, Vol. 16, pp. 291-293, 1996.**

The invited commentary (Wynder) and editorial (Gori) address the interpretation of weak epidemiologic associations. The proper interpretation of weak epidemiologic associations is central to the ETS component of the proposed rule. The Cal/EPA administrative record contains substantial evidence that weak associations are inherently unreliable and difficult to interpret.[64] The lower the

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[63]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 12-21; *see also,* Butler 1996a, pp. 29-37; OSHA Tr. at 1912 (Steenland) and 870 (Samet) (the potential for bias as an alternative explanation for an association increases as the level of observed risk declines, depending on the constellation of confounders); OSHA Tr. at 12444, 12510 (Ford) (the weaker the risk ratio, the greater the need to ensure that there are no biases or confounding).

[64]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 5-12; *see also,* Butler 1996a, pp. 29-37; OSHA Tr. at 1912 (Steenland) and 870 (Samet) (the potential for bias as an alternative explanation for an association increases as the level of observed risk declines, depending on the constellation of confounders); OSHA Tr. at 12444, 12510 (Ford) (the weaker the risk ratio, the

magnitude of an epidemiologic association, the greater the concern that bias from multiple sources (including confounding) can explain the entire association.[65]

Wynder and Gori make very similar points regarding the issues. Gori addresses the uncertainty inherent in weak statistical associations:

> [L]oose judgmental criteria have been introduced to give causal appearance to statistical associations: strength, consistency, specificity, temporality, response gradient, plausibility, coherence, and analogy. Such criteria are inadequate for an analytical evaluation of how uncertain causal inferences might be, and they fail to address the structural uncertainties derived from biases and confounders.

Gori 1996, p. 293. Wynder similarly concludes that, "[t]rue associations of this order [less than 2.0] are more likely to be affected by classification biases, confounding, case or control selection, and selective subgroup analysis than would be the case for large order associations." Wynder 1996, p. 747.[66]

Because it is uncontroverted in the record that all of the studies on ETS, lung cancer and heart disease produce, at most, "weak" associations, Cal/EPA must carefully apply the principles discussed by Gori and Wynder in interpreting these issues.

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greater the need to ensure that there are no biases or confounding).

[65]Even OSHA has repeatedly acknowledged that the strength of the association is a critical criterion in the decision of whether to infer, or not to infer, causation: "Thus, there is a crucial difference between the interpretation of positive and negative studies: studies that show no association, or only a weak positive association, need scrupulous review to exclude the possibility of confounding factors . . . ." 45 Fed. Reg. 5043 (1980).

[66]*See* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 7-9; *see also* OSHA Tr. at 14982 (Bayard) ("a factor of two to a risk assessor is peanuts").

9. ***Random Effects Methods in Meta-Analysis with Application in Epidemiology*, Biggerstaff, B.J.; In partial fulfillment of the requirements for the degree of Doctor of Philosophy, Colorado State University, Fort Collins, Colorado, Spring, 1995.**

Biggerstaff discusses random effects meta-analysis and uses the unadjusted ETS lung cancer data set as the main example. He clearly states that his purpose is to investigate statistical methods and not to analyze these data. Therefore, Biggerstaff's "conclusions" about the ETS data are not evidence upon which Cal/EPA can rely.[67] Biggerstaff's discussion of meta-analytic methodology, however, should be considered by Cal/EPA in its analysis of the ETS lung cancer data.

Biggerstaff discusses the need for "sensitivity" analysis before placing trust in meta-analysis results and concludes that sensitivity analysis "may reinforce or cast into doubt conclusions drawn from standard random effects inferences." According to Biggerstaff, sensitivity analysis mandates that the authors of a study check the accuracy of all of their assumptions:

> [We] utilize statistical models to describe the underlying association between lung cancer and ETS. These are formulated under certain modeling assumptions, and the conclusions drawn in inference using these models are only as strong as these assumptions are valid. When applying these methods, the meta-analyst is urged to carry out checks of assumptions made during model formulation, and these checks we call *sensitivity analyses*.

Biggerstaff 1995, p. 76.

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[67]The ETS data Biggerstaff uses are not adjusted for any confounders (including age). Biggerstaff does not account for publication bias in the female studies (although he acknowledges evidence of it) nor does he account for possible effects of other biases such as smoking status misclassification. Thus, the results of Biggerstaff's analysis should be accorded no weight by Cal/EPA.

Biggerstaff explains that the classical "logit" analysis, the type of analysis used in the epidemiology studies relied upon by both U.S. EPA and Cal/EPA, under-estimates the confidence interval (CI) width by 5-10% compared to a Fisher exact CI:

> [T]he supposed 95% CIs produced by the logit and test-based approximations are perhaps 5-10% narrower than the exact CIs for the sample sizes in these [spousal ETS] studies, with more extreme differences of almost 20% for studies based on very small cell numbers. . . . This indicates that considerable care must be taken in interpreting "marginally significant" results based on these approximations. . . . [B]oth the logit and test-based CI estimates for the individual [workplace] studies are again approximately 3-5% optimistic (that is, narrower), so more likely to find "significant" results spuriously on occasion.

Biggerstaff 1995, pp. 35, 38. This issue is not considered further in Biggerstaff's analyses nor was it considered in the Cal/EPA analyses. Thus, Cal/EPA's reliance on logit analyses increases the likelihood that the agency's assumptions regarding ETS and lung cancer are not as stable as they appear to be.

10. ***Misclassification of Smoking Habits as a Source of Bias in the Study of Environmental Tobacco Smoke and Lung Cancer*, Lee, P.N., Forey, B.A.; Statistics in Medicine, Vol. 15, pp. 581-605, 1996.**

Lee and Forey review the misclassification of smoking status literature and address the impact of misclassification biases on ETS and lung cancer studies.

a. When the magnitude of an association is weak, addressing the role of misclassification bias is critical

Lee and Forey identify the obstacles inherent in efforts to interpret weak epidemiologic associations:

> Our paper emphasizes the difficulties of interpreting weak associations in epidemiology, and the need to quantify the various sources of bias before coming to a firm conclusion. Though there are difficulties in quantifying the exact effects of smoking habit misclassification, our analyses show its likely importance and cast doubt on simple interpretation of the association between lung cancer and spousal smoking as indicative of a cause and effect relationship.

Lee and Forey 1996, p. 603.[68]

b. Misclassification biases may lead to an apparent relationship when no true relationship exists

Lee and Forey explain how misclassification of smoking status spuriously elevates risks attributed to ETS: "providing there is positive concordance between spouses' smoking habits, random misclassification of some subjects who smoke as non-smokers leads to an apparent relationship of spousal smoking to risk in non-smokers when no true relationship exists." Lee and Forey 1996, p. 586.[69]

c. The U.S. EPA report underestimates the impact of bias due to smoking status misclassification

Lee and Forey review the U.S. EPA report on ETS regarding the impact of smoking status misclassification bias and provide an update to the U.S. EPA's analysis with more current studies based on assumptions similar to those used in the U.S. EPA report. Lee and Forey find that EPA's misclassification adjustment is deficient in a number of ways that cause the bias introduced by smoking status misclassification to be underestimated:

> The recent EPA report considered data from 11 U.S. studies, with an RR estimate of 1.22 (95 per cent CI 1.04-1.4) reducing to 1.19 (95 per cent CI 1.01-1.38) after adjustment. Assuming a 1.75 per cent misclassification rate, and a concordance ratio of 3.0, our analysis reduces an RR of 1.13 (95 per cent CI 1.01-1.27) to 1.05 (95 per cent CI 0.93-1.18). There are three main reasons why our misclassification adjusted RR is much lower than that of EPA, and non-significant: (i) our analysis includes three studies not considered by EPA, one a large study. Overall the three studies show no association with spousal smoking (RR = 1.01; 95 per cent CI 0.82-1.23); (ii) the individual study data we used give slightly lower RRs. . . . Thus our estimate

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[68] *See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 5-12.

[69] *See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 26-28 (a proper analysis of misclassification bias demonstrates that misclassification bias may be producing the entire observed elevation of risk).

> for the same 11 studies EPA used is 1.20 (95 per cent CI 1.05-1.38) rather than 1.22; (iii) the actual bias estimated by EPA, of 1.03 is substantially lower than we estimate, 1.08, using apparently similar assumptions.

Lee and Forey 1996, pp. 599-600.

d. The Wells model that is the basis of U.S. EPA's misclassification adjustment is unreliable

Lee and Forey point out flaws in the Wells misclassification model used by U.S. EPA to adjust for misclassification bias: the Wells model "depends on many inadequately explained and justified assumptions and imprecisely known parameters," and has errors in the method. Lee and Forey 1996, pp. 588-589.[70]

e. The misclassification rates used by U.S. EPA are too low

Lee and Forey point out that there are numerous problems in the EPA determination of concordance ratios and misclassification rates. For concordance ratios, Lee and Forey show that the four publications cited by EPA in support of their estimates of concordance ratios contain either no or very little relevant data:

> As support for these estimates, EPA cited four references, but gave no detailed data. In fact, the first reference gives no relevant data at all, the second only gives data relevant to a different concordance ratio, while the third only gives data relevant to the first of the three concordance ratios . . . . Only the fourth reference gives a joint breakdown of smoking habits of subjects and spouses by never/ex/current status and even that was estimated indirectly.

Lee and Forey 1996, p. 596 (citations omitted).

For misclassification rates, Lee and Forey show that the EPA estimates are poorly justified and too low. Lee and Forey present data suggesting that the true ever smoker misclassification rate is likely nearer 2.5% (compared to the EPA-equivalent 1.75% rate). An EPA-equivalent rate of

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[70] *See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 23-25.

1.75% reduces the RR to 1.05, but a more appropriate 2.5% rate reduces the RR to 1.0. Lee and Forey 1996, p. 597.[71]

Lee and Forey conclude that "these analyses are consistent with the slight excess lung cancer risk observed in relation to smoking by the husband, being due not to ETS exposure, but to an artefact arising from misclassification of some smokers as non-smokers." Lee and Forey 1996, p. 598.[72]

11. *Familial Risk of Lung Cancer Among Nonsmokers and Their Relatives*, Schwartz, A.G., Yang, P., Swanson, M., American Journal of Epidemiology, Vol. 144, pp. 544-562, 1996.

Schwartz *et al.* report an association between lung cancer in nonsmokers and family history of lung cancer. "The traditional case-control comparison demonstrated that nonsmokers with lung cancer were 40 percent more likely than nonsmokers to report a positive family history of lung cancer in a first-degree relative." Schwartz *et al.* 1996, p. 558. Schwartz *et al.* also report that, after adjustment for ETS exposure and other factors, "among nonsmoking females [with a first-degree family history of lung cancer], a 1.7-fold increased risk was observed (95 percent CI 0.9-3.3)." Schwartz *et al.* conclude that "our findings demonstrate that the greatest contribution of family history to lung cancer risk among nonsmokers occurred in subjects 40-59 years of age who had a

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[71]Cal/EPA's record contains substantial evidence that smoking status misclassification rates are actually higher than 2.5%. *See* Oak Ridge National Laboratory ("ORNL") 1995, p. 31 (finding smoking status misclassification rates ranging from 3.0 to 6.0 percent); Ogden 1995a, p. 4, Appendix A, pp. 21-22 (finding smoking status misclassification rates ranging from 2.81 to 4.1 percent); Butler 1996b, p. 1 (calculating genders-combined misclassification rates for recent users of tobacco from self-reported never- and former-users as: 5.6% (all subjects); 5.0% (married subjects); and 5.9% (currently employed subjects). *See also*, RJR 1996 Cal/EPA Lung Cancer Comments, pp. 26-27 (citing ORNL 1995; Ogden 1995a; and Butler 1996b for the above calculations).

[72]*See also* RJR 1996 Cal/EPA Lung Cancer Comments, pp. 23-28.

sevenfold increased risk [RR=7.2, 95% CI: 1.3-39.7]" and that "this suggests a strong genetic component to lung cancer." The Schwartz *et al.* paper therefore provides further evidence that family history is a potential confounder for studies attempting to analyze ETS and lung cancer, especially in the female spousal ETS studies.

Schwartz *et al.* also provide risk estimates for lung cancer and home ETS exposure (OR = 1.1; 95% CI: 0.8-1.6) and for lung cancer and work ETS exposure (OR = 1.5; 95% CI: 1.0-2.2). These estimates are adjusted for age, sex and race but, unlike the family history results, are not adjusted for any of the other lung cancer risk factors identified in the paper. Many of these risk factors, like family history, have odds ratios for lung cancer that are much greater in magnitude than ETS exposure (*e.g.*, COPD: OR = 4.3; Tuberculosis: OR = 2.1; Emphysema: OR = 1.9; Allergies: OR = 0.5). Failure to adjust for these risk factors limits the reliability of the odds ratios for ETS exposure and lung cancer.

12. New Epidemiologic Studies on ETS and Lung Cancer

RJR submits the following table and supporting ETS and lung cancer literature as an addition to Tables 7.4-7.7 of the 1997 Draft to reflect new ETS epidemiologic studies that Cal/EPA must evaluate as it revises the lung cancer chapter of its 1997 Draft.

Additions to Tables 7.4-7.7

Study/location	Gender	ETS Exposure	Cases/Controls	OR (95% CI)*
Choi et al. (1989) /Korea	F	Spouse	75/164	1.63 (0.92-2.87)
	M		13/96	2.73 (0.49-15.2)
Jöckel (1991) /Germany	F	Partner	23/45	2.27 (0.75-6.82)
	M		9/70	2.68 (0.58-12.4)
Du et al. (1993) /China	F	Husband	75/254	1.09 (0.64-1.85)
Liu et al. (1993) /China	F	Husband	38/69	1.66 (0.73-3.78)
Layard (1994) /USA	F	Spouse	39/1930	0.58 (0.30-1.13)
	M		21/998	1.47 (0.55-3.94)
Zaridze and Zemlyanaya (1994) /Russia	F	Husband	162/285	1.66 (1.12-2.46)*
		Family member		1.08 (0.67-1.74)*
		Workplace		1.23 (0.74-2.06)*
		Childhood		0.98 (0.66-1.45)*
Cardenas (1997) /USA	F	Spouse	150 of 192,234	1.2 (0.8-1.8)*
	M		97 of 96,532	1.1 (0.6-1.8)*
Cardenas (1995)		Workplace	?	dose-level only
Wang et al. (1995) /China	F	Husband	135/135	1.11 (0.67-1.84)
		Workplace		0.89 (0.46-1.73)
		Childhood		0.91 (0.56-1.48)
Schwartz et al. (1996) /Detroit	F&M	Home	257/277	1.1 (0.6-1.6)*
		Workplace		1.5 (1.0-2.2)*
Shen et al. (1996) /China	F	?	70/?	0.85 (0.26-2.74)
Sun et al. (1996) /China	F	Husband	230/230	1.16 (0.80-1.69)*

Study/location	Gender	ETS Exposure	Cases/Controls	OR (95% CI)*
Wang et al. (1996) /China	F	?	?/?	2.5 (1.3-5.1)
Ko et al. (1997) /China	F	Spouse	105/105	1.3 (0.7-2.5)*
		Cohabitant		1.0 (0.4-2.3)*
		Workplace		1.1 (0.4-3.0)*
		Childhood		0.8 (0.4-1.6)*

Note: * indicates adjusted OR and CI

E. Chapter 8: Cardiovascular Effects

Chapter 8 (Cardiovascular Health Effects) of Cal/EPA's 1997 Draft reviews 17 epidemiologic studies of ETS and cardiovascular disease. In Appendix C to these comments, Dr. Carr Smith and Patricia DeLuca illustrate a major methodologic limitation of the epidemiologic studies of cardiovascular disease and ETS. Dr. Smith and DeLuca's comments establish the following points:

- The accuracy in calculating a dose-response is critical in assessing risk in an epidemiologic study.
- Patient records, death certificates, and interview data are inadequate measures of "response", *i.e.*, incidence of cardiovascular disease.
- Epidemiologic studies of living patients diagnosed with coronary artery disease are subject to selection bias because the majority of individuals with coronary artery disease remain undetected.
- An autopsy is necessary to determine accurately if the cause of death was due to cardiovascular disease.
- In the absence of an autopsy, death certification leans toward a diagnosis of coronary artery disease.
- Socioeconomic factors tend to increase the tendency toward the artifactual certification of coronary artery disease in the spouses of smokers.

- The epidemiologic studies of ETS and cardiovascular disease likely suffer from a diagnostic bias that tends to overestimate the CVD risk in nonsmoking spouses of smokers.

In summary, all 17 epidemiologic studies of ETS and cardiovascular disease suffer from a significant methodologic deficiency of selection/diagnostic bias that creates variability and uncertainty in the reported results. These epidemiologic studies depend on inadequate measures of cardiovascular disease incidence by relying on death certificates, medical records, and interviews (patients and/or next-of-kin) to determine the cause of death of study subjects.[73] An autopsy is the only method of classification that can accurately establish a cause of death. Because inaccurate diagnoses are more likely to occur in patients who are married to smokers (Cal/EPA's chosen surrogate for spousal ETS exposed subjects), the results reported in the 17 epidemiologic studies likely overestimate the risk of cardiovascular disease in nonsmoking spouses married to smokers.

Cal/EPA must consider, analyze, and address this new information and its impact on the certainty of its conclusions concerning ETS and cardiovascular disease in its revisions to Chapter 8 of the 1997 Draft.

IV. THE 1997 DRAFT TABLES CONTAIN NUMEROUS ERRORS AND PROVIDE STATISTICAL RESULTS THAT MUST BE CORRECTED OR EXPLAINED

A. Table 7.4 Comments

- The table does not include Layard (1995), Cardenas et al. (1997) and Schwartz et al. (1996). Layard reports results from the 1986 National Mortality Followback Survey (NMFS), Cardenas et al. report results from the American Cancer Society's Cancer Prevention Study II (CPS-II), and Schwartz et al. report results from Detroit.

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[73]One study, Svendsen *et al.*, 1987, did use a small percentage of autopsy reports to certify the diagnosis of cardiovascular disease, but the vast majority of causes of death were classified according to hospital/physician records, death certificates, and next-of-kin interviews.

- The entry "ethnicity," under "Matching variables of lifetime nonsmoking controls" in Fontham (1994) is not appropriate. The Fontham published papers indicate that matching was based on "race" only. The study was originally designed to use only English-language subjects but after the study was underway and case ascertainment had begun, its protocol was changed to add subjects whose primary language was either Chinese or Spanish. Thereafter, primary language was used in matching. The failure to match on the other aspects of ethnicity, particularly concerning the Chinese subjects, is a documented flaw in the study. *See* Steichen, 1995.

- The table reports that 100% of the cases in Fontham (1994) were confirmed by independent histologic review. The published paper reports this rate as only 85%.

- The entry under "Biologic markers" for Fontham fails to indicate that only 53.5% of cases and 83.3% of controls were tested for urinary cotinine, that cotinine cannot be detected for smoking activity that occurred more than three to five days ago, and that smokers with lung cancer are likely to quit smoking as symptoms become apparent. Thus, the use of cotinine for excluding subjects may negatively impact the quality of the study by preferentially excluding smokers from controls but not from cases.

- The table fails to indicate that analyses are available (by Butler) for the Brownson (1992) study. These analyses provide results for self-respondents only that are limited to never-smokers. Instead, the table indicates a 34% self-respondent rate for nonsmokers and ex-smokers combined. Use of Butler's analyses shows the effect of using self- versus proxy-respondents. *See* Butler, 1995.

B. Table 7.5 Comments

- The table does not include Layard (1995), Cardenas et al. (1997) and Schwartz et al. (1996). Layard reports results from the 1986 National Mortality Followback Survey (NMFS), Cardenas et al. report results from the American Cancer Society's Cancer Prevention Study II (CPS-II), and Schwartz et al. report results from Detroit.

- The reported results for Fontham (1994) have not been analyzed correctly for the reported interaction of childhood and adulthood exposure. *See* Butler, 1996.

C. Table 7.6 Comments

- The table does not include Zaridze & Zemlyanaya (1994), Wang et al. (1995), Sun et al. (1996), and Ko et al. (1997).

- **Janerich *et al.***: The table lists the number of cases among unexposed as 37 but the paper lists this as 57. A crude OR and CI for exposed versus unexposed can be computed as 1.30 (95% CI: 0.85-2.00).

- **Fontham *et al.***: Should not be used until the interaction issue identified by Butler is resolved. *See* Butler, 1996. The table lists the count of unexposed controls for father smoked in childhood as 699 but the paper lists this count as 669. The table lists the OR for 18+ years of childhood household exposure as 0.89, but the paper lists this as 0.88.

- **Kabat and Wynder**: The OR and CI listed in the table were not provided in the paper.

- **Kabat**: The results listed [incorrectly as Kabat *et al.*, 1990, the cite should be Kabat, 1990] were superseded by Kabat *et al.*, 1995. This entry should be deleted.

- **Wu-Williams *et al.***: The table lists the unexposed-case count for father smoked as 335 but the paper lists it as 235. The CI's listed in the table were not in the paper.

- **Pershagen *et al.***: The study is listed in the table as a 1986 paper but is a 1987 paper. The exposure surrogate is listed in the table as "parents smoked" but is given as "at least 1 parent smoked" in the paper. Case counts for exposed ("yes") and unexposed ("no") are reversed in the table. The paper lists 9 exposed and 38 unexposed (not 38 and 9, respectively). The table lists counts for controls of 76 exposed and 18 unexposed. The paper does not provide these counts nor are they consistent with the published CI (they are consistent with the OR).

D. Table 7.7 Comments

The following errors are present in Table 7.7:

- The table does not include Zaridze & Zemlyanaya (1994), Cardenas (1995), Wang et al. (1995), Schwartz et al. (1996), Sun et al. (1996), and Ko et al. (1997).

- **Kabat and Wynder**: Upper CI limit is 10.6, not 10.4 as shown in table.

- **Janerich *et al.***: Reported as "no association" in table but OR and CI are available in the paper as 0.91 (95% CI: 0.80-1.04).

- **Brownson *et al.***: Reported as "no association overall" in table but Butler 1995 provides OR and CI as 0.9 (95% CI: 0.7-1.15) for all employed subjects and 1.1 (95% CI: 0.8-1.7) for self respondents.

- **Fontham *et al.***: Should not be used until the interaction issue identified by Butler, 1996, is resolved.

- **Kalandidi *et al.***: OR and CI in table are inappropriate crude estimates comparing risk for housewives (as unexposed controls) versus workplace ETS exposure. This comparison is invalid. The paper provides an estimate of 1.08 (95% CI: 0.24-4.87) for a comparison of "extreme quartiles" of workplace ETS exposure.

- **Koo *et al.***: The study is listed in the table as a 1987 paper but is a 1984 paper. (The 1987 paper updated the spousal exposure results only; the workplace results were not updated or even reported in the 1987 paper). The 1984 paper provides the 0.91 crude estimate listed in the table but also provides the counts (not listed in the table) so that the crude CI can be calculated and listed. The counts are 22 unexposed and 2 exposed cases, and 40 unexposed and 4 exposed controls. These counts yield the 0.91 OR and a 95% CI of 0.15-5.37.

- **Shimizu *et al.***: The table does not list a CI but the paper provides the 95% CI as 0.69-2.01.

- **Wu-Williams *et al.***: The table lists the exposed-case count as 230 but the paper lists it as 228. The table lists the OR as 1.2 but the paper reports it as 1.1.

V. ISSUES RAISED BY RJR IN PREVIOUS COMMENTS TO CAL/EPA

A. RJR's Comments on the 1996 Lung Cancer Draft Chapter

RJR's April 1, 1996 comments presented an overview of the topics discussed in Cal/EPA's 1996 Lung Cancer Draft Chapter and provided a bibliography of relevant sources of information.[74]

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[74]RJR's 1996 comments provided Cal/EPA with a concise analysis of the flaws in the 1996 Draft, as well as the numerous defects and limitations in the U.S. EPA 1992 report on which Cal/EPA relies. RJR's 1996 comments served as a guide to literature that Cal/EPA should review. The 1996 comments included citations to original papers, review articles, and submissions by RJR and others to federal agencies (*e.g.*, U.S. EPA and OSHA) that have considered or are considering similar issues. It is incumbent that Cal/EPA review all of the citations to RJR's 1996 comments and

RJR's 1996 comments focused on: (1) the inadequacy of the defective U.S. EPA 1992 report as a benchmark for Cal/EPA's analysis; (2) the inconsistency between the U.S. EPA's 1992 conclusions and the four post-1991 U.S. epidemiologic studies reviewed in the 1996 Draft (Brownson *et al.*, 1992, Fontham *et al.*, 1994, Kabat *et al.*, 1995, and Stockwell *et al.*, 1992); and (3) specific issues raised by the epidemiologic studies. Cal/EPA includes an appendix in its 1997 Draft which, according to Cal/EPA, "summarizes written comments received from the public during the formal comment periods, as well as responses to those comments." (1997 Draft, Preface, p. ii.) Cal/EPA's Appendix A does not acknowledge receipt of or respond to any of the many issues and additional information provided in RJR's 1996 comments. Rather than simply repeat RJR's 1996 comments on the 1996 Lung Cancer Draft Chapter in their entirety, RJR here provides brief summaries of the points raised in its 1996 comments and attaches a copy of its 1996 comments in Appendix D. The following sections describe briefly each of the issues raised and additional evidence presented in RJR's 1996 comments to which Cal/EPA has provided no response.

1. The U.S. EPA (1992) report is a defective benchmark for Cal/EPA's analysis of ETS and Lung Cancer.

In the 1997 Draft, Cal/EPA recognizes that the U.S. EPA 1992 report has numerous shortcomings.[75] Nonetheless, the 1997 Draft adopts the U.S. EPA approach without performing a

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all the citations to these comments, including the materials reviewed therein, to identify the best available evidence on the questions before the Agency and to understand fully the analysis presented in these comments.

[75]Cal/EPA recognized that studies on which U.S. EPA based its assessment of ETS and lung cancer had numerous weaknesses, including small sample size, possible selection bias, possible misclassification bias of nonsmoker status and disease status, inadequate adjustment for potential confounders, inadequate measurement of ETS exposure, publication bias, difficulty of obtaining adjusted risk estimates, and general methodologic weaknesses. Cal/EPA 1997 Draft, pp. 7-12 to 7-13, 7-25.

critical reassessment or further development of its approach or methodology. By relying on the conclusions reached by the U.S. EPA (1992) report, Cal/EPA's 1997 Draft promulgates the U.S. EPA's faulty analysis and methodology. Among its numerous flaws, the U.S. EPA 1992 report:

- ignores the problems inherent in interpreting weak associations,
- uses improper one-sided tests of significance and 90% confidence intervals,
- employs an improper approach to assessing confounders and other biases,
- reaches conclusions that do not account for the uncorrected biases and confounders,
- employs inconsistent relative risk selection criteria,
- mixes U.S. and foreign studies,
- mixes disease endpoints,
- mixes exposure measures, and
- performs inappropriate analyses (trend tests, highest dose, binomial count analyses, and meta-analysis).

In its 1996 comments, RJR provided many citations to scientific evidence that support these points. (RJR 1996 Comments, pp. 4-49).

a. U. S. EPA ignored the fundamental problems inherent in interpreting the weak and inconsistent associations reported in the ETS and lung cancer studies.

(RJR 1996 Comments, pp. 5-12).

Cal/EPA should not repeat the mistakes of the U.S. EPA. In its evaluation of the epidemiologic data, Cal/EPA should proceed from the perspective that epidemiologic studies are notoriously unreliable for interpreting weak associations. Weak associations, such as the associations reported in the ETS epidemiologic literature, must be reviewed rigorously and viewed skeptically. Moreover, Cal/EPA's efforts to assess confounding and bias should focus on the small

magnitudes of association reported for ETS and lung cancer. Cal/EPA must carefully assess the large number of confounders and biases that might explain the small associations reported in the ETS epidemiologic studies.

b. U.S. EPA incorrectly analyzed confounding in the epidemiologic studies by employing an improper definition of confounding, ignoring most of the relevant data, failing to assess joint confounding, and failing to quantify the amount of bias introduced by confounding.

(RJR 1996 Comments, pp. 12-22).

Confounding of data is one of the chief limitations in interpreting weak epidemiologic associations. Cal/EPA must recognize that the published ETS epidemiologic studies do not evaluate ETS exposure. Instead, they evaluate "spousal smoking status," "parental smoking status," and/or "co-worker smoking status." Thus, the risk evaluated is the risk associated with the full constellation of factors associated with such "statuses." Spousal smoking status is correlated highly with numerous recognized or suspected risk factors for lung disease. Consideration of confounding is critical in analyzing epidemiologic studies of ETS.

U. S. EPA employed an improper definition of confounding. (RJR's 1996 Comments, pp. 13-14). U.S. EPA incorrectly claimed that "[c]onfounding requires the presence of a non-ETS cause of lung cancer associated with ETS exposure." U.S. EPA, 1992, p. 5-16. Fundamental principles of epidemiology provide that a potential confounder need not be a "cause" of a disease. It is necessary only that the factor under consideration as a potential confounder be associated with the exposure (or, for ETS, the exposure surrogate) of interest. Next, the U.S. EPA rationalized that the observed risk could be attributable to confounding only if there existed a single confounder that explained the full elevation in risk in every study from every country. U.S. EPA, 1992, p. 5-63.

U.S. EPA claimed that it could find no such single confounder that would fully explain the risk in all countries and studies and, therefore, concluded that confounding could be ruled out. U.S. EPA, 1992, p. 1-10. There is no requirement that a single confounder need be the source of all reported associations of ETS to lung cancer. ETS exposure is associated with a large number of factors, each of which could contribute to the distorted measure of association. U.S. EPA's assertion that it is sufficient to rule out each potential confounder individually as a source of the entire amount of potential confounding is erroneous.

U.S. EPA's failure to consider external data in its confounding analysis is inconsistent with standard epidemiologic practice. (RJR 1996 Comments, pp. 15-17). The vast majority of the epidemiologic studies cited by U.S. EPA did not collect the data required to perform an adequate examination of confounding. When epidemiologic studies do not thoroughly evaluate confounding directly, standard epidemiologic principles require the performance of an independent assessment of confounding using external data to assess confounding. However, the U.S. EPA restricted its review of confounding to six limited categories of confounding using only the results from 31 epidemiologic studies of spousal smoking and lung cancer. In doing so, EPA ignored the large amount of data from sources other than the spousal smoking studies that were brought to the Agency's attention. The technique employed by U.S. EPA indicates a lack of familiarity with basic principles of epidemiology or with the epidemiologic literature on the dietary, health history, occupation, lifestyle, and environmental risk factors for lung cancer. EPA provided no analysis of how it determined that the spousal smoking epidemiologic studies are a sufficient database for analyzing confounding. Failure to use external information, when the investigators did not collect

information, to assess directly the presence of confounding is equivalent to assuming that no confounding is present.

U.S. EPA failed to consider numerous sources of confounding in spousal epidemiologic studies. (RJR 1996 Comments, pp. 17-21). For lung cancer, these potential confounding factors include multiple dietary factors, physical activity, socioeconomic status (SES), alcohol consumption, age, occupational exposures to other carcinogens and irritants, asbestos exposure, exposure to non-workplace carcinogens, personal history of lung disease (including tuberculosis), use of various cooking fuels, personal medical care (including drug treatment), family history of lung cancer and lung disease (genetics), home environment (type of housing), lifestyle, urbanization, current marital status, and use of illicit drugs. According to standard epidemiologic practice, it is necessary to consider all potential confounders if one is to obtain a valid estimate of the magnitude of an association. (RJR 1996 Comments, p. 21).

U.S. EPA failed to perform an analysis of the joint effects of potential confounding factors. (RJR 1996 Comments, pp. 21-22). The U. S. EPA incorrectly asserted that a single confounder must explain the full elevation in risk in every study in order to justify the conclusion that associations between ETS and lung cancer observed in epidemiologic studies are not attributable to confounding. U. S. EPA, 1992, p. 5-63. Multiple confounders could introduce a magnitude of bias in one study that is greater than that introduced by any single confounder alone. Therefore, the assessment of confounding must be executed in a multivariate manner with consideration of numerous factors. OEHHA must expand its consideration of confounding to include a multivariate assessment of confounding. (RJR 1996 Comments, p. 22).

In summary, available data shows that differences in lifestyle and other factors exist between never smoking women married to smokers and never smoking women married to nonsmokers. Using the available data on the increased risk for some of these factors, it has been shown that observed associations in the range of 1.10 readily can be explained by just one of these confounders. The confounding that results from the joint effect of these factors could be much greater. Because of the many flaws in U.S. EPA's analysis of confounding, Cal/EPA should not rely on the report as its benchmark for the consideration of the impact of confounding factors on the ETS epidemiologic studies.

c. U. S. EPA underestimated the influence and uncertainty of smoking status misclassification bias on the epidemiologic studies by employing a model that ignores statistical variability in the input parameters and by basing its analyses on the limited and biased subset of the then-available data.

(RJR 1996 Comments, pp. 23-28).

Cal/EPA has acknowledged that smoking status misclassification bias is an important bias in the epidemiologic studies. Cal/EPA 1997 Draft, pp 7-20 to 7-21. The 1997 Draft purports to account for smoking status misclassification bias by adopting the misclassification approach set forth in the U.S. EPA (1992) report and wrongly concludes that the four additional U.S. epidemiologic studies identified in Cal/EPA's analysis have, collectively, corrected and controlled for this bias.

Cal/EPA should not rely on the misclassification adjustment in the U.S. EPA (1992) report. The adjustment is based on a convoluted model (the "Wells model") with numerous flaws. (RJR's 1996 Comments, pp. 24-25).

There is abundant new data concerning smoking status misclassification (ORNL, 1995; Ogden 1995; Butler NHANES III) that is of higher quality and inconsistent with that used by the U. S. EPA. These data demonstrate that smoking status misclassification bias alone is sufficient to nullify the claims of increased lung cancer risk in pooled epidemiologic studies. (RJR's 1996 Comments, pp. 26-27).

The post-1991 epidemiologic studies have done nothing to resolve the role of misclassification of smoking status. The Stockwell and Brownson studies did not even attempt to address the issue. Fontham *et al.* used a misguided approach that introduced additional bias. To properly assess the role of smoking status misclassification, Cal/EPA must conduct an independent examination of the best, most representative data and must use a model that is statistically valid.

d. U. S. EPA's analysis is affected by publication bias

(RJR 1996 Comments, pp. 29-34).

Every meta-analysis, including the U.S. EPA's meta-analysis, depends on the assumption that: 1) all relevant studies have been included or 2) the sample of available studies is representative of all studies. Because of the critical nature of this assumption, it has become standard practice to formally consider how publication bias might influence the conclusions reached in a meta-analysis. U.S. EPA's analysis ignores the well-accepted principle that publication bias exists. (RJR 1996 Comments, pp. 29-31).

The published literature on ETS and health shows evidence of publication bias. In fact, there are a number of remarkable examples of instances in which non-positive results were not published by the original investigators (e.g., Layard 1995 (NMFS); LeVois & Layard, 1995 (CPS

I, II); Brownson *et al.*, 1992; Stockwell *et al.*, 1992, Fontham *et al.*, 1994). (RJR 1996 Comments, pp. 31-33).

The 1997 Draft fails to properly address the issue of publication bias, instead claiming that "[t]he issue of publication bias has been reviewed in detail by Bero *et al.*, 1994." The Bero study used highly unorthodox and unscientific methods to gather information regarding unpublished ETS literature. An examination of the Bero article reveals that no true attempt was made to discover publication bias in the ETS literature. Instead, the authors searched routine medical databases, which by their very nature would not contain unpublished results, and by asking tobacco industry sources about unpublished medical studies -- a source least likely to be advised of such studies.

Bero *et al.* present no discussion of the literature that addresses appropriate methods for studying publication bias. The Bero study dismisses the study by LeVois and Layard (1995) which provided a detailed review of the methods and history of publication bias and identified three studies (datasets) that were not in the published literature on ETS. (LeVois, April 17 OEHHA Workshop, Tr. at 54-56).

e. U. S. EPA's analyses do not rule out chance as an explanation for the statistical association reported

(RJR 1996 Comments, pp. 34-43).

Only statistically significant results provide potentially reliable evidence of an association. The test of statistical significance is crucial to any analysis of epidemiologic data. Without this test, artifactual associations produced by chance (sampling variation) are more likely to be misinterpreted. In effect, statistical significance tests are used as a threshold screening analysis. Associations that are not statistically significant provide no basis for scientific conclusions or

administrative actions. Associations that are statistically significant pass this threshold test and can be evaluated further by other standard criteria. (RJR 1996 Comments, p. 34-35).

U.S. EPA (1992) analyzed thirty-one worldwide epidemiologic studies of spousal smoking and lung cancer. Of the thirty-one studies reviewed by EPA, eleven were conducted in the United States. As originally reported, none of the U.S. studies were statistically significant at the 95% level for the overall summary risk estimate.[76] Furthermore, after U.S. EPA corrected the studies for smoking status misclassification, only one of the eleven was statistically significant even at the 90% confidence level. Of the thirty-one worldwide studies reviewed by U.S. EPA (1992), twenty-four of the studies were not statistically significant as originally reported for the overall risk estimate. Even after U.S. EPA reanalyzed all worldwide studies using 90% confidence intervals, over two-thirds of the studies were not statistically significant for the overall risk estimate. (RJR 1996 Comments, p. 35).

U.S. EPA use of 90% confidence intervals is inconsistent with standard epidemiological practices. The standard test for statistical significance is a two-tailed test with $\alpha = 0.05$, and is often expressed as a 95% confidence interval. Switching to a one-tailed test for statistical significance doubled the likelihood of labeling as "statistically significant" an association observed by chance. (RJR 1996 Comments, p. 36).

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[76]Fontham *et al.*, 1994, was not published prior to the release of the U.S. EPA (1992) report.

f. U.S. EPA employed less rigorous statistical trend-test analyses to evaluate dose-response because the traditional, and more rigorous, tests employed by statisticians provide results inconsistent with U.S. EPA's conclusions.

The U.S. EPA's "dose-response" analysis consists of the examination of statistical tests for trends for those studies that either report the results of the trend tests or provide crude data that permit those tests to be performed. The trend test used by U.S. EPA is known as the "Cochran Armitage trend test" or the "Mantel-Haenszel extension test for trend." The analysis depends on a number of critical assumptions, including that:

- the foreign studies are of equal weight and relevance as the U.S. studies;
- the studies for which "dose"-level data and/or trend-test results were published are representative of all of the studies in the U.S. EPA (1992) report;
- it is meaningful to perform trend-test analyses on unadjusted data;
- the trend test "dose" levels should be equally spaced;
- the zero-dose (unexposed) level should be included in the trend test analysis;
- a linear trend implies dose-response;
- it is appropriate to convert the tests of significance for a linear trend from two-sided tests to one-sided tests; and
- the higher "doses" represent a true dose level.

Only four of the current fifteen U.S. spousal smoking lung cancer studies provide statistically significant trend-test results. These results were obtained via a test that does not test dose-response. The test was applied to the wrong (unadjusted) data. Arbitrary "dose" levels were assigned. The original studies did not report, nor measure, true doses, and the results differed dramatically when the zero-dose level was excluded. Therefore, Cal/EPA should place no weight on U.S. EPA's analysis of dose-response. (RJR's 1996 Comments, pp. 37-40).

U.S. EPA's highest potential exposure analysis is misleading. The "highest dose" analysis in the U.S. EPA report consists of examining reported relative risks for the highest "exposure" category in each of the studies. This analysis is also flawed because it depends on numerous unsubstantiated assumptions, including many of the same assumptions as the "dose-response" analysis. (RJR 1996 Comments, pp. 40-41).

U. S. EPA's binomial count analyses are meaningless. In these analyses, U.S. EPA counts the number of "significant" results generated by their "dose-response" or "highest-dose" analyses, and then calculates the "probability" of the number of statistically significant results occurring by chance. There are significant flaws in the U.S. EPA's "binomial count" analyses. First, the "binomial count" methodology requires that all measures be independent binomial trials. Second, U. S. EPA's "binomial count" analyses assume that each study carries equal weight in assessing risk. Third, U.S. EPA applies the "binomial count" analyses only to those studies that provide the data necessary to perform the "dose response" and "highest dose" analyses, not to the full set of studies. The "probabilities" calculated, are therefore, biased upward by the exclusion of the other studies. Fourth, U.S. EPA counts a study that reports results for more than one measure of "exposure" as significant in the binomial count analysis if any of the multiple results are significant, thus disregarding the internally inconsistent nonsignificant result(s) and improperly including a study with an aberrant risk estimate. Finally, U.S. EPA's binomial count analyses are based on dose-response and highest-dose analyses of unadjusted data. Therefore, the analyses do not account for sources of bias and confounding. (RJR 1996 Comments, pp. 41-43).

g. U. S. EPA's use of meta-analysis to summarize the ETS epidemiologic studies was improper

(RJR 1996 Comments, pp. 43-47).

The epidemiologic studies of ETS and lung cancer are too heterogeneous to combine in a meta-analysis. The individual ETS epidemiologic studies differ significantly in study quality, design, location, definition of exposure, adjustments for confounding, and other characteristics. Methods of meta-analysis are intended only for highly similar studies, a condition not present in the ETS epidemiology studies. (RJR 1996 Comments, pp. 44-46).

U. S. EPA included only a biased subset of the available data in its meta-analysis. U.S. EPA manipulated and cherry-picked the data that it included in its meta-analysis. For example, U.S. EPA completely ignored two large studies published in the United States, Stockwell (1992) and Brownson (1992), despite the fact that these studies were brought to the U.S. EPA's attention prior to release of the 1992 report. The Brownson (1992) study shows no overall association between ETS and lung cancer, and the Stockwell (1992) study does not report a statistically significant increased risk estimate between ETS and lung cancer. Further, U.S. EPA chose to include the interim results of the Fontham study while ignoring the interim results of the Kabat study. Likewise, U.S. EPA did not acquire and include the data from the 1986 National Mortality Followback Survey. These data also showed a nonsignificant reduced lung cancer risk for females. U. S. EPA's actions biased the analysis and invalidated the conclusions of its meta-analysis of the ETS epidemiologic studies. (RJR 1996 Comments, pp. 46).

The EPA-style of meta-analysis of data now available is inconsistent with U. S. EPA's conclusions. (RJR 1996 Comments, pp. 46-47).

2. Epidemiologic research since 1992 contradicts U.S. EPA's conclusions

(RJR Comments, pp. 49-70).

Cal/EPA's 1997 Draft reviews four post-1991 epidemiologic studies of lung cancer and ETS. The four post-1991 studies provide data that, when properly analyzed, contradict the conclusions reached by the U.S. EPA. The Brownson *et al.*, 1992 study is a negative study. The most reliable data in the Brownson study -- the direct respondents -- show no increase in risk for spousal or workplace exposure, including the highest exposure groups. The published Fontham *et al.*, 1994 report is unreliable because of flaws in the study design and interpretation. Moreover, when properly analyzed, even the flawed Fontham data shows no association between reported adult ETS exposure and lung cancer risk. The Kabat *et al.*, 1995 study has important design improvements over the other studies. As the investigators accurately stated regarding their results, "the pattern of odds ratios shows little indication of an association between ETS and lung cancer in non-smokers." Stockwell *et al.* report no statistically significant increased risks and no risk at all for workplace exposure despite failing to address confounding and bias. (RJR 1996 Comments, p. 3).

a. The Brownson 1992 data show no association between ETS and lung cancer risk.

(RJR 1996 Comments, pp. 51-54)

As reported by Brownson *et al.*, 1992, there is no overall elevated lung cancer risk for lifetime nonsmokers exposed to spousal ETS. Cal/EPA 1997 Draft, p. 7-16. Specifically, for all lifetime nonsmoking subjects exposed to spousal ETS, the adjusted odds ratio (exposed vs. unexposed) is 1.0 (95% CI: 0.8-1.2). (RJR 1996 Comments, p. 51).

Cal/EPA recognizes that analyses of surrogate interview data are not as reliable as analyses of direct interview data. Cal/EPA 1997 Draft, pp. 7-21. In Brownson, the results for the direct interviews show no significant association for either workplace or household exposure. Butler,

1995, Tables 2 and 5a. As Dr. William J. Butler explained at both the March 26, 1996 and April 17, 1997 public workshops, the increased risk of lung cancer risk reported by Brownson *et al.* for those with > 40 pack-years of household exposure occurs only among those with a surrogate interview and not among those with direct interviews. (RJR 1996 Comments, pp. 51-53) (Butler, April 17 OEHHA Workshop, Tr. at 92-94).

Brownson *et al.* find no elevated lung cancer risk associated with workplace ETS exposure. Specifically, for all nonsmoking subjects who worked outside the home for at least six months, the adjusted odds ratio (exposed vs. unexposed at work) is 0.98 (0.74-1.31). (RJR 1996 Comments, pp. 53-54)

Brownson *et al.* report no association between risk of lung cancer and ETS exposure from parents (OR=0.7, 95% CI: 0.5-0.9) or other household members (OR=0.8 95% CI: 0.6-1.1) during childhood. Cal/EPA 1997 Draft, p. 7-22, Table 7.6. (RJR 1996 Comments, p. 54).

b. The published Fontham *et al.*, 1994 report is unreliable

(RJR 1996 Comments, pp. 54-62).

The ostensible quality of the Fontham *et al.*, 1994 publication is a chimera. Examination of the study design and execution reveal that the results are unreliable:

- The Fontham study population is not representative of the U.S. population nor of California.
- The technique used by Fontham to detect misclassification of smoking status is insufficient, is inadequately performed and leads to a bias that elevates the observed risk estimate.
- The reported OR's in Fontham 1991 for colon-cancer controls are uniformly smaller than analogous estimates for population controls, suggesting recall bias. Nevertheless, the colon cancer controls were discontinued.

- The use of frequency-only matching within age categories, combined with the high sensitivity of cancer incidence to age differences, likely introduces a bias resulting in inflated risk estimates.

- Data on important confounders, such as alcohol and fat consumption, were not collected.

- Fontham's use of an inappropriate reference group introduces a recognized, but uncorrected bias, that inflates the apparent relative risk for adulthood exposures.

- The percentage of adenocarcinoma cases is unusually high and may reflect abnormal demographics in the study population.

- The result of Fontham's adjustment for confounders for the workplace data is in the opposite direction from that expected and is inconsistent with other adjustments in the same study.

- Exposure levels are indirect and imprecise and exposure levels are poorly defined.

- The absence of dose from the "risk equation" necessitates reliance upon the recall of exposures that took place many years ago, often by surrogate respondents.

- The non-independence of the spousal-, workplace- and social-exposure study populations forces Fontham's workplace relative risk estimate to include potential contributory effects (including confounding) from both spousal and social settings.

(RJR 1996 Comments, pp. 54-62).

c. Properly analyzed, the Fontham *et al.* data show there is no association between adult ETS exposure and lung cancer risk

(RJR 1996 Comments, pp. 62-68).

Dr. William J. Butler has shown that if the Fontham data are properly analyzed, the data show no association between adult ETS exposure and lung cancer. Dr. Butler shows that a critical, statistical interaction exists between childhood exposure and adulthood exposure in the Fontham data. (Butler, April 17 OEHHA Workshop, Tr. at 90-92). The interaction results in an apparent, but spurious elevation in lung cancer risk for those study subjects who report exposure both in childhood and in adulthood. When the proper reference group (i. e., those exposed in neither childhood nor

adulthood) is used for the Fontham data, the apparent increased risk for those exposed in both childhood and adulthood disappears. (RJR 1996 Comments, pp. 63-64).

The significance of this finding by Butler for interpreting the Fontham data cannot be overstated:

- Never smoking women with both adult and childhood ETS exposure are at no greater risk of lung cancer than never-smoking women with neither adult nor childhood exposure (OR = 1.00; 95% CI = 0.61, 1.64).

- Never-smoking women with adult but no childhood ETS exposure are at no greater risk of lung cancer than never-smoking women with neither adult nor childhood exposure (OR = 1.00; 95% CI = 0.60, 1.67).

- Combining the two results stated above, among never-smoking women, adult ETS exposure is not associated with an increased risk of lung cancer, regardless of the presence of childhood ETS exposure.

- Never-smoking women with childhood but no adult ETS exposure are at a significantly lower risk of lung cancer than never-smoking women with neither adult nor childhood exposure (OR = 0.35; 95% CI = 0.12, 0.99). This statistically significant negative association is most likely an artifact of bias either in study design or data collection.

(RJR 1996 Comments, pp. 64-65).

d. Kabat *et al.*, 1995 is inconsistent with U.S. EPA's conclusions

(RJR 1996 Comments, pp. 68-69).

The recently published Kabat *et al.* study is inconsistent with U.S. EPA's conclusions. Kabat *et al.* report a RR of 1.08 (95% CI: 0.60-1.94) for females and 1.60 (95% CI: 0.67-3.82) for males in a case-control study of U.S. subjects. The gender-combined RR is 1.22 (95% CI: 0.75-1.99). While computation of a gender-combined value is legitimate, it is inappropriate to compare it to the female-only EPA estimate, as Cal/EPA has done. The appropriate comparison estimate from Kabat *et al.* is the 1.08 female-specific value.

e. Stockwell *et al.*, 1992 is inconsistent with U.S. EPA's conclusions

(RJR 1996 Comments, pp. 69-70).

Stockwell *et al.* report a statistically nonsignificant spousal exposure RR of 1.60 (95% CI: 0.8-3.0). In addition, the authors report "no statistically significant increase in risk associated with environmental tobacco smoke exposure at work...." Stockwell *et al.*, 1992, p. 1420. The nonsignificant spousal-exposure and workplace-exposure findings in this large and recent U.S. study confirm the negative results of the Brownson study.

3. Studies of workplace ETS exposures do not demonstrate an increased lung cancer risk

(RJR 1996 Comments, pp. 70-97).

During the March 25, 1996 public workshop on the 1996 Lung Cancer Draft Chapter, Dr. Anna Wu emphasized that Cal/EPA was interested in examining non-spousal ETS exposure as a secondary objective. Dr. Wu stated that although non-spousal exposures are more difficult to measure and produce less reliable risk estimates, epidemiologic studies of non-spousal exposures have produced "supportive evidence." Dr. Wu is wrong on both counts. First, there is no valid basis to conclude that spousal studies provide a more reliable risk estimate than workplace studies. Second, the workplace studies provide no basis for concluding that workplace exposure increases lung cancer risk. These issues are addressed in great detail in the docket for OSHA's Proposed Rule on Indoor Air Quality. Cal/EPA should examine closely the information contained in OSHA's docket on these issues. (RJR 1996 Comments, pp. 70-71).

To address the question of whether workplace ETS exposure is associated with an elevated risk of lung cancer, Cal/EPA should critically examine the entire group of available workplace

studies. All but Fontham (1994)[77] (as reported by the authors) fail to show a significant association of workplace ETS exposure with lung cancer and must be considered non-positive. Fontham reports results for adulthood exposure that have been shown to be based on an inappropriate comparison that inflates the reported relative risks. Consequently, there is no reliable data that supports the conclusion that workplace ETS exposure presents a risk of lung cancer.

As reported by Brownson *et al.*, there is no elevated lung cancer risk associated with workplace ETS exposure. Specifically, for all nonsmoking subjects who worked outside the home for at least 6 months, the adjusted odds ratio (exposed vs. unexposed at work) is 0.98 (0.74-1.31). (RJR 1996 Comments, pp. 81-83).

Stockwell *et al.* report "no statistically significant increase in risk associated with environmental tobacco smoke exposure at work...." Stockwell *et al.*, 1992, p. 1420. The authors did not elaborate further, nor did they provide the supporting data and summary results for this conclusion on workplace exposure. The negative workplace-exposure finding in this recent U.S. study confirms the negative results of the Brownson study. (RJR 1996 Comments, pp. 84-85).

The published Fontham workplace results suffer from numerous and serious problems that render those results inappropriate as a source of information for Cal/EPA's assessment. *See supra*, pp. 75-76. As Dr. Butler explained during both the March 25, 1996 and the April 17, 1997 public workshops, the published Fontham results are biased by a statistical interaction that inflates the apparent relative risks. The statistical interaction in the data was not corrected in the analyses

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[77]Kabat and Wynder (1984) reported a marginally significant positive association for the male subgroup and a nonsignificant negative association for the larger female subgroup. The study is not significant when the genders are combined.

published by Fontham *et al.*, thus rendering all reported results unreliable and unusable. (RJR 1996 Comments, pp. 85-87) (Butler, April 17 OEHHA Workshop, Tr. at 90-92).

Dr. Butler reanalyzed the Fontham (1994) data to demonstrate and quantify the bias present in that study. Butler's reanalysis was necessarily limited to a single subset of the data, the adulthood exposure to ETS from all environments (home, social and work), because Fontham *et al.*'s published reports provide only limited and selected data. This single subset is the only data sufficiently described to allow a reanalysis. (RJR 1996 Comments, pp. 86).

The best (and only correctly analyzed) evidence from the Fontham study shows no increase in risk from ETS exposure. Although these results are not workplace-specific, workplace ETS exposure is a component of the adulthood exposures analyzed by Butler. For workplace-specific exposures, a more complete analysis of the bias resulting from the statistical interaction documented by Butler requires access to the raw Fontham data. Cal/EPA should insist that the data from the Fontham study be made available for reanalysis by Cal/EPA and other parties.[78] Otherwise, Cal/EPA must either reject the Fontham study or must use the best evidence provided by Butler. (RJR 1996 Comments, pp. 86-87).

To date, nine ETS and lung cancer studies have collected data for exposure in U.S. workplaces. Most of the studies are limited to female subjects; only Butler (1988), Janerich (1990), Kabat and Wynder (1984), and Kabat (1995), included male subjects. The U.S. studies, as a group, do not support the inference that workplace ETS exposure is associated with an increased risk of

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[78]Cal/EPA should be particularly interested in acquiring the Fontham data due to the direct involvement of the author of the Carcinogenic Effects chapter of the 1997 Draft, Anna Wu, as the investigator in charge of the Los Angeles component of the study. It is noteworthy that Anna Wu was not present at the April 17, 1997 public workshop to respond to Dr. Butler's analysis, nor has Cal/EPA included any meaningful response to Dr. Butler's findings in Chapter 7 or Appendix A.

lung cancer. Table 2 of RJR's 1996 Comments summarizes the data from the workplace studies. (RJR 1996 Comments, pp. 87-97).

B. RJR's October 1995 Comments on Exposure Measurements and Prevalence

RJR's October 16, 1995 comments presented an overview of the topics discussed in Cal/EPA's 1995 ETS Exposure Measurements and Prevalence Draft Chapter and provided a bibliography of relevant sources of information.[79] However, Cal/EPA has apparently chosen to respond to the numerous criticisms of Chapter 2 by simply changing the objective of Chapter 2: "This chapter provides *background information* on the prevalence and measurement of exposure to ETS, and emphasizes investigation and monitoring methods used in epidemiological evaluations of health effects." Cal/EPA 1997 Draft, p. 2-1 (emphasis added).[80]

By simply revising the objective of Chapter 2, Cal/EPA demonstrates its failure to understand that exposure assessment and epidemiology are complementary processes in risk assessment. Epidemiology without exposure assessment conveys little, if any, meaning about the health implications of ETS exposure for the citizens of California. By relegating exposure assessment to "background information" status, the Agency has perverted the science of risk

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[79]RJR's 1995 comments provided Cal/EPA with a concise analysis. The 1995 comments included citations to original papers, review articles, and submissions by RJR and others to federal agencies (*e.g.*, U.S. EPA and OSHA) that have considered or are considering similar issues. It is incumbent that Cal/EPA review all of the citations to RJR's 1995 comments and all the citations to these comments, including the materials reviewed therein, to identify the best evidence on the questions before the Agency and to understand fully the analysis presented in these comments.

[80]In 1995 Cal/EPA stated that the purpose of the Exposure chapter was to focus on exposure issues relevant to the health effects addressed throughout the draft risk assessment: "This chapter summarizes available information on exposure to environmental tobacco smoke (ETS), with a focus on exposure relevant to health effects considered in other chapters of the overall ETS assessment." Cal EPA, 1995, p. v.

assessment. The Agency should revise Chapter 2 to address exposure assessment adequately. To allow Chapter 2 to remain in its current form within the document would be scientifically irresponsible, since such would mislead the public as well as the scientific community about hazard assessment (specifically, epidemiology), exposure assessment, and risk assessment.

In RJR's October 16, 1995 comments, RJR demonstrated that, in order to produce a document that adequately describes what is known about the magnitude and prevalence of ETS exposures, Cal/EPA must address the following fundamental questions:

1. What is meant by the term "exposure"?

2. What is the best methodology for assessing ETS exposure?

3. What substances provide the best quantitative estimates when measured as "markers" of ETS exposure? and

4. What are the best available data for estimating current levels of ETS exposure and the prevalence of those exposures in the United States in general and California in particular?

Chapter 2 of Cal/EPA's 1997 Draft, however, is materially identical to the Agency's 1995 ETS Exposure Measurements and Prevalence Draft Chapter and fails to address many of the critical issues raised by RJR's 1995 Comments. Although Appendix A of the 1997 Draft attempts to respond to some of the concerns raised by RJR, the Appendix ignores the majority of RJR's 1995 Comments. Moreover, the responses in Appendix A are superficial responses to extremely complex and critical issues.

Cal EPA's revision of Chapter 2 indicates that the Agency's response to comments is arbitrary and that it is engaging in publication bias. RJR provided the Agency with a substantial number of scientific papers and publications on ETS exposure assessment. Some of these represented the state-of-the-art on the subject. The revised Chapter 2 contains not a single one of

these state-of-the-art publications. Furthermore, the revised chapter fails to acknowledge by literature citation that these exist. The Agency's total rejection of key scientific information dealing directly with ETS exposures indicates that Cal/EPA's response is arbitrary.[81]

Rather than simply repeat RJR's 1995 comments on the 1995 ETS Exposure Measurements and Prevalence Draft Chapter in their entirety, the following section will describe briefly each of the issues raised and additional evidence presented in RJR's 1995 Comments to which Cal/EPA provided no response.[82]

1. Cal/EPA's attempt to assess the chemical and physical properties of ETS is misleading and inappropriate

a. Cal/EPA should not rely on mainstream smoke and sidestream smoke data to assess ETS

Mainstream smoke ("MS") and sidestream smoke ("SS") are precursors of ETS, rather than components. Because of dilution and aging, ETS is a complex mixture distinct from either SS or MS in terms of physical and quantitative chemical properties. As Cal/EPA notes at p. 2-2 of the 1997 Draft Exposure Chapter, during the aging and dilution process, SS undergoes physical and chemical changes, including changes in particulate size and composition. The use of the properties

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[81]RJR notes that Cal EPA did choose to add a few publications to its revised Chapter 2. Two of these include Crawford *et al.* (1994) (Cal/EPA 1997 Draft, p. 2-31) and Denissenko *et al.* (1996) (Cal/EPA 1997 Draft, p. 2-32). Neither publication satisfies Cal/EPA's stated reason for rejecting consideration of several key studies on ETS exposure that RJR submitted. Rather, both of these publications discuss "markers [that] have not been widely adopted by other researchers in the field." (Cal/EPA 1997 Draft, p. 2-11). Moreover, both involve exposure assessment methods that "have only been used in a limited way for measuring ETS concentrations in real world environments." (Cal/EPA 1997 Draft, p. 2-11). The Agency employs a double standard which indicates that Cal EPA is "cherry-picking" the scientific literature to support a predetermined position.

[82] Where appropriate, this section also will demonstrate the inadequacy of Cal/EPA's cursory attempt - in Appendix A - to respond to some of the concerns previously raised by RJR.

of one, the other, or both of these precursors to predict the properties of ETS is incorrect. (Rodgman, 1992, 1994, 1995).

As a result of this aging and dilution process, the ETS to which a nonsmoker is exposed is an extremely dilute system compared to MS inhaled by a smoker or fresh, whole SS. Concentrations of substances in ETS can be several orders of magnitude less than concentrations of the same substances in MS or SS.

Moreover, Cal/EPA should not attempt to describe ETS by discussing MS/SS ratios for various constituents. MS/SS ratios are not relevant to assessing ETS for two main reasons. First, as Cal/EPA recognizes, MS to SS ratios are highly variable and can be misleading. (Cal/EPA 1997 Draft, p. 2- 4). This variability is even greater for MS/ETS ratios. Second, MS/SS ratios have little or no meaning when applied to real world environments. A MS/SS constituent ratio conveys no information about the absolute concentration of the constituent in either MS or SS -- much less ETS.

b. Cal/EPA's review of ETS constituents should be eliminated

Cal/EPA's review of "Biologically Active Constituents of ETS" contained in Section 2.2.2 of the 1997 Draft is uninformative, misleading and should be eliminated.[83] First, the potential biologic activity of a complex mixture cannot be predicted based on a review (in this case, a partial and incomplete review) of the constituents believed to be contained in the mixture. Second, many of the constituents discussed by Cal/EPA have never been measured in ETS at levels found in real world environments. (Rodgman, 1992). Third, Chapter 2 does not quantify those ETS constituent

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[83] In Appendix A, Cal/EPA responds that Chapter 2 is designed to provide background information on exposures and does not reach conclusions regarding toxicity. (App. A, p. A-50). Cal/EPA, however, fails to recognize that its identification of several of these constituents does not provide any information relevant to exposure because the identified constituents have never been measured in real world environments.

levels that have been in real world environments. Fourth, Chapter 2 contains no discussion of the conditions under which the constituents have been shown to have biologic activity (e.g., at what dose, route of administration, duration of administration, species tested and endpoint(s) examined, etc.) and consequently fails to show the relevance of the constituent's reported biologic activity to ETS exposure.

Concentrations of these substances in ETS are orders of magnitude less than concentrations deemed as acceptable by reputable authorities. For example, Gori and Mantel (1991) estimated the number of cigarettes required to yield concentrations that reach the threshold limit values (TLV) estimated by the American Conference of Governmental and Industrial Hygienists in 1990 for eight substances. The space was assumed to be sealed, unventilated, and 100-m^3 in volume. For the eight components, their estimates were: cadmium -- 1430 cigarettes; acetaldehyde -- 1430 cigarettes; benzene -- 13,300 cigarettes; nickel -- 40,000 cigarettes; hydrazine -- 145,000 cigarettes; benzo[a]pyrene -- 222,000 cigarettes; 2-toluidine -- 300,000 cigarettes; and polonium-210 -- 750,000 cigarettes. Holcomb 1994 (Table 8) provides comparisons of ETS constituent levels to corresponding OSHA permissible exposure limits.

2. Assessment of ETS exposure

Cal/EPA's Chapter 2 still demonstrates a lack of understanding by Cal/EPA of fundamental issues related to assessing ETS exposure. Because of this lack of understanding, the Chapter continues to be incomplete, adequately uninformative, and misleading. Exposure is correctly defined simply as concentration times time ($E = C \times T$). As this product shows, two pieces of information are needed to quantify exposure; namely, a concentration level and a duration. Cal/EPA has failed to recognize this concept throughout its Draft Chapter by using the term "exposure" to

refer to concentration levels without reference to duration and to potential durations without reference to concentration levels.[84]

Additionally, to develop a meaningful chapter on ETS exposure assessment, Cal/EPA needs to address two fundamental concepts:

1. What qualities must a marker of ETS exposure possess? and

2. What is the best approach to assessing ETS exposure?

a. Cal/EPA fails to give proper consideration to criteria that assess a marker's usefulness for determining ETS exposure

The National Research Council and others have addressed the qualities that a good exposure marker should possess.[85] Cal/EPA, however, has failed to recognize any of these criteria and instead emphasizes the use of airborne nicotine and RSP as ETS exposure markers. Studies employing

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[84]In Appendix A, Cal/EPA claims to recognize this flaw but misses the point by responding incorrectly that information on duration or concentrations alone can assist in classifying individuals into exposure categories (high or low). Appendix A p. A-50.

[85]The NRC identifies four qualities which an ETS exposure marker should possess.

> A marker or tracer for quantifying ETS concentrations should be:
>
> (1) unique or nearly unique to the tobacco smoke so that other sources are minor in comparison,
>
> (2) a constituent of the tobacco smoke present in sufficient quantity such that concentrations of it can be easily detected in air, even at low smoking rates,
>
> (3) similar in emission rates for a variety of tobacco products, and
>
> (4) in a fairly consistent ratio to the individual contaminant of interest (e.g., suspended particulates) under a range of environmental conditions encountered and for a variety of tobacco products.

NRC, 1986, p. 70.

markers such as nicotine and RSP have been superseded by better evidence. In assessing ETS exposures, Cal/EPA should restrict its attention to studies employing the best markers, *i.e.* 3-EP for the ETS vapor phase and - in order of preference - solanesol, FPM and UVPM for the ETS particle phase.[86]

Cal/EPA also emphasizes the use of biomarkers for assessing ETS exposures. Although Chapter 2 recognizes the severe limitations in using thiocyanate, carbon monoxide or carboxyhemoglobin as markers of ETS exposure,[87] Cal/EPA still emphasizes the use of biologic cotinine levels to measure ETS exposure. The presence of cotinine in sera or other biologic fluids, however, does not quantify ETS exposure because: 1) cotinine can originate from dietary sources of nicotine and 2) measurable levels of cotinine can occur because of exposure to ETS-nicotine that exists in the absence of measurable levels of other ETS constituents. In Appendix A to the 1997 Draft, Cal/EPA recognizes that biologic cotinine is useful in identifying active smokers among self-reported nonsmokers. Cal/EPA fails to recognize, however, that cotinine cannot quantify ETS exposure.

b. Personal Monitoring is the Proper Approach for Assessing ETS Exposure

Personal monitoring is the only reliable means of determining an individual's actual ETS exposure. Through personal monitoring, information can be obtained on ETS concentrations and duration of exposures for the particulate and vapor phases.

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[86]See discussion in Section III.A., supra; see also RJR's October 16, 1995 Comments, pp. 14-24.

[87]See 1997 Draft Exposure Chapter, pp. 2-17 - 2-18.

By contrast, other techniques such as area monitoring and use of biomarkers or questionnaires are inferior to personal monitoring. Whereas area monitors provide information only on concentration at the site of the monitor, questionnaires provide information only on potential duration of exposure. With respect to biomarkers, none of the biomarkers which have been used to date satisfy the NRC criteria.

3. Current ETS exposures in the United States

In addition to the many methodological problems already identified, the studies of exposure that Cal/EPA has relied upon are not representative of current exposures. Results from current studies that are representative of the United States, including California, and that employed personal monitoring in conjunction with reliable markers of ETS, show that ETS exposures are substantially lower than reported by Cal/EPA.

Cal/EPA simply ignored the two recent ETS exposure studies provided by RJR in its October 16, 1995 Comments, *i.e.* the ORNL and Ogden *et al.* studies. Both of these studies have since been published and have been provided to Cal/EPA as peer-reviewed and published studies.[88] ORNL studied nonsmoker ETS exposure in 16 U.S. cities, including one in California. This study is the most comprehensive and most representative assessment of ETS exposure done to date. Ogden *et al.* employed procedures similar to those of ORNL reports actual exposures that are at least an order of magnitude less than implied by the concentration range listed by Cal/EPA.[89]

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[88]See Section III.A.12 and III.A.13, *supra*.

[89]Each of these studies are described in great detail in RJR's October 16, 1995 Comments. pp. 24-31. Rather than reiterate their findings here, RJR recommends that Cal/EPA review these studies and include them in any discussion the agency attempts to provide regarding actual ETS exposures. Failure to do so would be arbitrary and capricious.

4. Misclassification of smoking status

Cal/EPA properly recognizes the importance of misclassification of smoking status: "[m]isclassification of an individual who is a smoker as a nonsmoker may increase the apparent relative risk of smoking-related diseases in nonsmokers." (Cal/EPA 1997 Draft, p. 2-22.) In addition, Cal/EPA recognizes that the biomarker cotinine can be used to distinguish between smokers and nonsmokers: "[a] number of studies have used biomarkers to validate self-reported smoking status." (Cal/EPA 1997 Draft, p. 2-22.) The Agency summarizes results from some studies published in 1992 and earlier examining smoking status misclassification in Table 3.6. However, Cal/EPA appears to dismiss the effect of smoking status misclassification when it quotes Perez-Stable: "most misclassified smokers are very light smokers or occasional smokers who binge." (Cal/EPA 1997 Draft, p. 33). The Agency does not report the factual basis for Perez-Stable's supposition.

Proper adjustment for appropriate misclassification rates eliminates the small, weak, and inconsistent epidemiologic risks that have been reported for lung cancer rates.[90] Thus, Cal/EPA's apparent dismissal of the effect of smoking status misclassification is unfounded. In addition, Cal/EPA has relied upon studies that are not representative and underestimate misclassification rates. Recent studies representative of the United States, including California, show that misclassification rates are higher than previously reported.

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[90]For a detailed discussion of these issues, see RJR's October 16, 1995 Comments, pp. 39-44.

5. The prevalence of exposure to environmental tobacco smoke

Cal/EPA devotes a lengthy portion of Chapter 2 to a discussion of "exposure prevalence." It is unclear how this portion of Chapter 2 provides any information relevant to an assessment of ETS exposure for the California population. As RJR has already pointed out, exposure is best determined by measuring reliable markers of ETS with personal monitors over time relevant to exposures ($E = C \times T$). Cal/EPA's discussion of studies that for the most part present questionnaire results of persons claiming any ETS exposure for times ranging from the previous day to the previous week brings no scientifically useful information to a review of ETS exposure. This potential "prevalence" data contains no information on concentration and therefore, cannot quantify exposure. Cal/EPA must first define its purposes for discussing prevalence or at least state what its working definition of "prevalence" is.

BIBLIOGRAPHY

Alavanja, M. C. R. et al. "Saturated Fat Intake and Lung Cancer Risk among Nonsmoking Women in Missouri." *JNCI* 85: 1906-1916, 1993.

Baek, S.O., Kim, Y.S., Gee, I., "Estimation of ETS Contribution to RSP in Indoor Smoking Environments," *Indoor Air '96*, pp. 483-488, 1996.

Bayard, S., "Testimony of Steven Bayard, Ph.D., Project Manager, Passive Smoking Risk Assessment, U.S. Environmental Protection Agency, Before OSHA's Hearings on The Proposed Rule for Indoor Air Quality," March 10, 1995.

Bayard, S., "Testimony of Steven Bayard, Ph.D., Project Manager, Passive Smoking Risk Assessment, U.S. Environmental Protection Agency, Before OSHA's Hearings on The Proposed Rule for Indoor Air Quality," September, 1994.

Bero, L. A., Glantz, S. A., Rennie, D., "Publication Bias and Public Health Policy on Environmental Tobacco Smoke," *JAMA* 272: 133-136.

Biggerstaff, B. J., "Random Effects Methods in Meta-Analysis with Application in Epidemiology," In partial fulfillment of the requirements for the degree of Doctor of Philosophy, Colorado State University, Fort Collins, Colorado, Spring, 1995.

Bjørn, E., Nielsen, P.V., "Passive Smoking in a Displacement Ventilated Room," Presented at the 7th International Conference on Indoor Air Quality and Climate in Nagoya, Japan, July 21-26, 1996.

Blair, P. S., Fleming, P. J., Bentley, D., Smith, I., Bacon, C., Taylor, E., Berry, J., Tripp, J., "Smoking and the Sudden Infant Death Syndrome: Results from 1993-5 Case-Control Study for Confidential Inquiry into Stillbirths and Deaths in Infancy," *BMJ* 313: 195-198, 1996.

Blustein, J.,"High technology cardiac procedures. The Impact of Service Availability on Service Use in New York State," *JAMA* 270: 344-349, 1993.

Brownson, R. C., Alavanja, M. C. R., Hock, E. T., Loy, T. S., "Passive Smoking and Lung Cancer in Nonsmoking Women," *Am J Public Health* 82: 1525-1530, 1992.

Butler, W.J., "Prevalence of the Recent Use of Tobacco Among Self-Reported Never- and Former-Users of Tobacco: An Analysis of Misclassification Data From NHANES III," submitted to the Occupational Safety and Health Administration, February 9, 1996b.

Butler, W. J., "Re-Analysis of Data Provided in Fontham *et al.* (1994): No Increase in the Risk of Lung Cancer Associated with Adult ETS Exposure," submitted to the Occupational Safety and Health Administration, February 9, 1996c.

Butler, W. J., "Lung Cancer and Exposure to ETS in the Household and in the Workplace: Additional Analyses of the Data From a Negative Study, Brownson *et al.* (1992)," submitted to the Occupational Safety and Health Administration, September 1, 1995.

Butler, W.J., "Bias From Confounding Can Explain the Epidemiologic Association of EST With Lung Cancer and Heart Disease: Comments on Statements in the Record Regarding Confounding and Other Sources of Bias," submitted to the Occupational Safety and Health Administration, February 9, 1996a.

Cardenas, V. M., "Environmental Tobacco Smoke and Lung Cancer Mortality In the American Cancer Society's Cancer Prevention Study II," a dissertation submitted to the Faculty of the Graduate School of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Cardenas, W.M., Thun, M.J., Austin, H., Lally, C.A., Clark, W.S., Greenberg, R.S., Heath, C.W., "Environmental Tobacco Smoke and Lung Cancer Mortality in the American Cancer Society's Cancer Prevention Study II," *Cancer Causes and Control* 3: 57-64; 1997.

Carlisle, D. M., Leake, B.D., Shapiro, M.F., "Racial and Ethnic Difference in the Use of Invasive Cardiac Procedures among Cardiac Patients in Los Angeles County, 1986 through 1988," *Am J Public Health* 85: 352-356, 1995.

Coggins, C. R. E., Letter to the Editor Regarding: "Estimating Exposure to Environmental Tobacco Smoke," *JAMA* 276(8): 603, 1996.

Dalen, J. E., Alpert, J. S., "Natural History of Pulmonary Embolism," *Prog Cardiovasc Dis* 17: 259-270, 1975.

Delamothe, T., "Whose data are they anyway?," *BMJ* 312: 1261-1262, 1996.

Devine, M., Vickers, J., Liston, R., Banerjee, A.K., "How Well Does Mortality Reflect the Burden of Illness Due to Stroke?" *British Journal of General Practice* 46: 757, 1996.

Dismuke, S. E., Wagner, E. H., " Pulmonary Embolism as a Cause of Death: the Changing Mortality in Hospitalized Patients," *JAMA* 255: 2039-2042, 1986.

Domino, E.F., Letter to the Editor Regarding: "Estimating Exposure to Environmental Tobacco Smoke, *JAMA* 276(8): 603, 1996.

Du, Y.X., Cha, Q., Chen, Y.Z. and Wu, J.M., "Exposure to Environmental Tobacco Smoke and Female Lung Cancer in Guangzhou, China," *Proceedings of Indoor Air 1993*, Vol. 1: 511-516.

Emmons, K.M., Marcus, B.H., Abrams, D.B., Marshall, R., Novotny, T.E., Kane, M.E., Etzel, R.A., "Use of a 24-Hour Recall Diary to Assess Exposure to Environmental Tobacco Smoke, *Archives of Environmental Health* 51(2): 146-149, 1996.

Epstein, A. M., Taylor, W. C., Seage III, G. R., "Effects of Patients' Socioeconomic Status and Physicians' Training and Practice on Patient-Doctor Communication," *American Journal of Medicine* 78: 101-106, 1985.

Feinstein, A. R., Heterodemic studies. Chapter 24 in Clinical Epidemiology, The Architecture of Clinical Research , WB Saunders Company, Philadelphia, pp. 561-594, 1985.

Fontham, E. T. H., Correa, P., Reynolds, P., Wu-Williams, A., Buffler, P. A., Greenberg, R. S., Chen, V. W., Alterman, T., Boyd, P., Austin, D. F., Liff, J., "Environmental Tobacco Smoke and Lung Cancer in Nonsmoking Women," *JAMA* 271: 1752-1759, 1994.

Gatsonis, C. A., Epstein, A. M, Newhouse, J. P., Normand, S., McNeil, B. J., "Variations in the Utilization of Coronary Angiography for Elderly Patients with an Acute Myocardial Infarction," *Med Care* 33: 625-642, 1995.

Glantz, S.A., "Primer of Biostatistics," 3rd Edition, McGraw-Hill, Inc., New York, 1992.

Goldacre, M. J., "Cause-specific Mortality: Understanding Uncertain Tips of the Disease Iceberg," *J Epidemiol Community Health* 47: 491-496, 1993.

Goldhaber, S. Z., Hennekens, C. H., "Time Trends in Hospital Mortality and Diagnosis of Pulmonary Embolism," *Am Heart J* 104: 305-306, 1982.

Gori, G.B., "Epidemiology, risk assessment, and public policy: Restoring epistemic warrants," *Risk Analysis*, Vol. 16, pp. 291-293, 1996.

Gori, G.B., "Epidemiology, Risk Assessment, and Public Policy: Restoring Epistemic Warrants," *Risk Analysis* 16: 291-293, 1996.

Grant, H. D., Murray, R. H., Bergeron, J. D., Chapter 3 "Patient Assessment" in Brady Emergency Care, 5th Edition. Apprentice Hall Division, Englewood Cliffs , NJ. 1990, p. 75.

Guite, H. F., Burney, P. G. J., "Accuracy of Recording of Deaths from Asthma in the UK: the False Negative Rate," *Thorax* 51: 924-928, 1982.

Haglund, B., Cnattingius, S., Otterblad-Olausson, P., "Sudden Infant Syndrome in Sweden," *Am J Epidemiol* 142: 619-624, 1995.

Hodgson, A.T., Daisey, J.M., Mahanama, K.R.R., Ten Brinke, J., "Use of Volatile Tracers to Determine the Contribution of Environmental Tobacco Smoke to Concentrations of Volatile

Organic Compounds in Smoking Environments," *Environment International* 22(3): 295-307, 1996.

Hutchins, G., Personal communication with Grover Hutchins, M.D., Johns Hopkins Department of Pathology, May 1, 1997.

Idle, J., Benitez, J. Krokan, H.E., Lohman, P.H.M., Roberfroid, M., Springall, A., "Environmental Tobacco Smoke and Lung Cancer: An Evaluation of the Risk, Report of A European Working Group," April 1996.

International Agency for Research on Cancer (IARC), "IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Smoking," Vol. 38. Lyon, France: World Health Organization.

Janerich, D. T., Thompson, W. D., Varela, L. R.. Greenwald, P., Chorost, S., Tucci, C., Zaman, M., Melamed, M. R., Kiely, M., McKneally, M. F., "Lung Cancer and Exposure to Tobacco Smoke in the Household," *N Engl J Med* 323: 632-636, 1990.

Jockel, K.H., "Passive Smoking - Evaluation of the Epidemiological Findings," *Commission on Air Pollution of the VDI and DIN: Carcinogenic Substances in the Environment* (April 1991).

Kabat, G. C., Stellman, S. D., Wynder, E. L., "Relation between Exposure to Environmental Tobacco Smoke and Lung Cancer in Lifetime Smokers," *Am J Epidemiol* 142: 141-148, 1995.

Kabat, G. C., Wynder, E. L., "Lung Cancer in Nonsmokers," *Cancer* 53: 1214-1221, 1984.

Kalandidi, A., Katsouyanni, K., Voropoulou, N., Bastas, G., Saracci, R., Trichopoulos, D., "Passive Smoking and Diet in the Etiology of Lung Cancer among Non-Smokers," *Cancer Causes and Control* 1: 15-21, 1990.

Kannel, W. B., Schatzkin, A., "Sudden Death: Lesions from Subsets of Population Studies," *J Am Coll Cardiol* 5: 141B-9B, 1985.

Klonoff-Cohen, H. S., Edelstein, S. L., Lefkowitz, E. S., Srinivasan, I. P., Kaegi, D., Chang, J. C., Wiley, K. J., "The Effect of Passive Smoking and Tobacco Exposure through Breast Milk on Sudden Infant Death Syndrome," *JAMA* 273: 795-798, 1995.

Koo, L. C., Ho, J. H. C., Saw, D.C., Mok, C. K., "Is Passive Smoking an Added Risk Factor for Lung Cancer in Chinese Women?" *J Exp Clin Cancer Res* 3: 277-283, 1984.

Kuller, L. H., Shemanski, L., Psaty, B. M., Borhani, N. O., Gardin, J., Haan, M. N., O'Leary, D. H., Savage, P.J., Tell, G.S., Tracy, R., "Subclinical Disease as an Independent Risk Factor for Cardiovascular Disease," *Circulation* 92: 720-726, 1995.

Law, M.R., Hackshaw, A.K., "Environmental Tobacco Smoke," *British Medical Bulletin* 52(1): 22-34, 1996.

Layard, M. W., "Ischemic Heart Disease and Spousal Smoking in the National Mortality Followback Survey," *Regulatory Toxicology and Pharmacology* 21: 180-183, 1995.

Lee, P.N., Forey, B.A., "Misclassification of Smoking Habits as a Source of Bias in the Study of Environmental Tobacco Smoke and Lung Cancer," *Statistics in Medicine* 15: 581-605, 1996.

LeVois, M. E., Layard, M. W., "Publication Bias in the Environmental Tobacco Smoke/Coronary Heart Disease Epidemiologic Literature," *Regulatory Toxicology and Pharmacology* 21: 184-191, 1995.

LeVois, M. E., Layard, M. W., "Inconsistency between Workplace and Spousal Studies of Environmental Tobacco Smoke and Lung Cancer," *Regulatory Toxicology and Pharmacology* 19: 309-316, 1994.

Mackenbach, J. P., Looman, C. W. N., van der Meer, J. B. W., "Differences in the Misreporting of Chronic Conditions, by Level of Education: the Effect on Inequalities in Prevalence Rates," *American Journal of Public Health* 86: 706-711, 1996.

McGoon, M. D., "What is Heart Disease?" Chapter 2 in Mayo Clinic Heart Book. William Morrow and Company, New York; pp 70-71, 1993.

McIntosh, H. D., "Risk factors for Cardiovascular Disease and Death: a Clinical Perspective," *J Am Coll Cardiol* 14: 24-30, 1989.

Milerad, L., Rajs, J., Gidlund, E., "Nicotine and Cotinine Levels in Pericardial Fluid in Victims of SIDS," *Acta Paediatr* 83: 59-62, 1994.

Mitchell, E. A., Scragg, L., Clements, M., "Location of Smoking and the Sudden Infant Death Syndrome (SIDS)," *Aust NZ J Med* 25: 155-156, 1995.

Model, D., "Preventable Factors and Death Certification in Death Due to Asthma," *Respiratory Medicine* 89: 21-25, 1995.

Moolgavkar, S.H., Luebeck, E.G., "A Critical Review of the Evidence on Particulate Air Pollution and Mortality," *Epidemiology* 7: 420-428, 1996.

Morgenthaler, T. I., Ryu, J. H., "Clinical Characteristic of Fatal Pulmonary Embolism in a Referral Hospital," *Mayo Clinic Proceedings* 70: 417-424, 1995.

Moriyama, I. M., Dawber, T. R., Kannel, W. B., "Evaluation of Diagnostic Information Supporting Medical Certification of Deaths from Cardiovascular Disease," National Cancer Institute Monograph, 19: 405-419, 1966.

National Health Interview Survey, 1993. *Vital Health Stat* 10. December 1994 (190).

O'Sullivan, J. P., "The Coroner's Necropsy in Sudden Death: an Under-used Source of Epidemiological Information," *J Clin Pathol* 49: 737-740, 1996.

National Research Council (NRC), "Sharing Research Data," Feinberg, S.E., Martin, M.E., Straf, M.L. (eds.), National Academy Press, Washington, D.C., 1985.

National Research Council (NRC), "Environmental Tobacco Smoke: Measuring Exposures and Assessing Health Effects," National Academy Press, Washington, D.C., 1986.

Nilsson, R., "Environmental Tobacco Smoke and Lung Cancer: A Reappraisal," *Ecotoxicology and Environmental Safety* 34: 2-17, 1996.

Oak Ridge National Laboratories, "Post Hearing Comments: Proposed Rulemaking, Occupational Safety and Health Administration, 29 CFR Parts 1910, 1915, 1926, and 1928, Indoor Air Quality: Proposed Standard," submitted to the Occupational Safety and Health Administration, August 29, 1995.

Occupational Safety and Health Administration, Public Hearing on Proposed Standard for Indoor Air Quality, 1994-1995 (selected pages).

Ogden, M.W., "Comments on Environmental Tobacco Smoke Exposure," Post-Hearing Comments on the Occupational Safety and Health Administration's Notice of Proposed Rulemaking on Indoor Air Quality, September 1, 1995b.

Ogden, M.W., Letter to the Editor Regarding: "Estimating Exposure to Environmental Tobacco Smoke," *JAMA* 276(8): 603-604, 1996.

Ogden, M.W., Heavner, D.L., Foster, T.L., Maiolo, K.C., Cash, S.L., Richardson, J.D., Martin, P., Simmons, P.S., Conrad, F.W., Nelson, R.R., "Personal Monitoring System for Measuring Environmental Tobacco Smoke Exposure, *Environmental Technology* 17: 239-250, 1996.

Ogden, M. W., "Comments on Smoking Status Misclassification," submitted to the Occupational Safety and Health Administration, September 1, 1995a.

Pershagen, G., Hrubec, Z., Svensson, G., "Passive Smoking and Lung Cancer in Swedish Women," *Am J Epidemiol* 125: 17-24, 1987.

Phillips, K., Howard, D., Bentley, M., Alvan, G., "Assessment of Air Quality in Europe by Personal Monitoring of Nonsmokers in Homes/Workplaces for Respirable Suspended Particles (RSP), Environmental Tobacco Smoke (ETS), and Volatile Organic Compounds (VOCs) Inside and Outside Homes," Presented at the 7th International Conference on Indoor Air Quality and Climate in Nagoya, Japan, July 21-26, 1996.

Pirkle, J.L., Flegal, K.M., Bernert, J.T., Brody, R.A., Maurer, K.R., "Exposure of the U.S. Population to Environmental Tobacco Smoke: The Third National Health and Nutrition Examination Survey, 1988 to 1991," *JAMA* 275(16): 1233-1240, 1996.

Pirkle, J.L., Flegal, K.M., Letter to the Editor Regarding: "Estimating Exposure to Environmental Tobacco Smoke, *JAMA* 276(8): 604, 1996.

R. J. Reynolds Tobacco Company, "R. J. Reynolds Tobacco Company's Post-Hearing Brief," submitted to the Occupational Safety and Health Administration, February 9, 1996.

R. J. Reynolds Tobacco Company, "Comments of the R. J. Reynolds Tobacco Company on Environmental Tobacco Smoke: Exposure Measurements and Prevalence," submitted to the California Environmental Protection Agency, October 16, 1995.

R. J. Reynolds Tobacco Company, "Comments of the R. J. Reynolds Tobacco Company on Carcinogenic Effects of Exposure to Environmental Tobacco Smoke -- Excerpt: ETS and Lung Cancer," submitted to the California Environmental Protection Agency, April 1, 1996.

Reggio, A., Failla, G., Patti, F., "Reliability of Death Certificates in the Study of Stroke Mortality. A Retrospective Study in a Sicilian Municipality. *Ital J Neurol Sci* 16: 567-570, 1995.

Repace, J.L., Lowrey, A.H., "Letters to the Editor," *Environment International* 22(2): 268-270, 1996.

Robbins, S. L., Cotran, R. S., Kumar, V., Chapter 12 "Blood Vessels" in Pathologic Basis of Disease; WB Saunders Company, Philadelphia, 1984.

Robinson, J.P., Switzer, P., Ott, W., "Daily Exposure to Environmental Tobacco Smoke: Smokers vs. Nonsmokers in California, *AJPH* 86(9): 1303-1305, 1996.

Ross, J.A., Sterling, E., Collett, C., Kjono, N.E., "Controlling Environmental Tobacco Smoke in Offices," *HPAC* [Heating/Piping/Air Conditioning], pp. 76-83, 1996.

Rothlisberger, C., Meier, B., "Coronary Interventions in Europe 1992," *Eur Heart J* 16: 922-929, 1995.

Schecter, A. D., Glodschmidt-Cleremont, P. J., McKee, G., Hoffeld, D., Myers, M., Velez, R., Duran, J., Schulman, S. P., Chandra, H. G., Ford, D. E., "Influence of Gender, Race, and Education on Patient Preferences and Receipt of Cardiac Catheterization among Coronary Care Unit Patients," *Am J Cardiol* : 996-1001, 1996.

Schwartz, A.G., Yang, P., Swanson, M., "Familial Risk of Lung Cancer Among Nonsmokers and Their Relatives," *American Journal of Epidemiology*, 144: 544-562; 1996.

Sears, S.B., Steichen, T.J., "Comments on Environmental Tobacco Smoke: Preliminary Quantitative Risk Assessment," submitted to The Occupational Safety and Health Administration," August 13, 1994.

Shimizu, H., Morishita, M., Mizuno, K., Masuda, T., Ogura, Y., Santo, M., Nishimura, M., Kunishima, K., Karasawa, K., Nishiwaki, K., Yamamoto, M., Hisamichi, S., Tominaga, S., "A Case-Control Study of Lung Cancer in Nonsmoking Women," *Tohoku J Exp Med* 154: 389-397, 1988.

Sigurdsson, E., Gudmundur, T., Sigvaldason, H., Sigfusson, N., "Unrecognized Myocardial Infarction: Epidemiology, Clinical Characteristics, and the Prognostic Role of Angina Pectoris," *Ann Intern Med* 122: 96-102, 1995.

Steichen, T.J., "Post-Hearing Comments on Confounders," submitted to the Occupational Safety and Health Administration, September 1, 1995a.

Steichen, T. J., "Post-Hearing Comments on Non-English Speaking Chinese Women," submitted to the Occupational Safety and Health Administration, September 1, 1995c.

Sterling, T.D., Glicksman, A., Perry, H., Sterling, D.A., Rosenbaum, W.L., Weinkam, J.J., "An Alternative Explanation for the Apparent Elevated Relative Mortality and Morbidity Risks Associated with Exposure to Environmental Tobacco Smoke, *J Clin Epidemiol* 49: 803-808, 1996.

Sterling, E.M., Collett, C.W., Ross, J.A., "Assessment of Non-Smokers' Exposure to Environmental Tobacco Smoke Using Personal-Exposure and Fixed-Location Monitoring," *Indoor Built Environ* 5: 112-125, 1996.

Stockwell, H. G., Goldman, A. L., Lyman, G. H., Noss, C. I., Armstrong, A. W., Pinkham, P. A., Candelora, E. C., Brusa, M. R., "Environmental Tobacco Smoke and Lung Cancer Risk in Nonsmoking Women," *J Natl Cancer Inst*, 84: 1417-22, 1992.

Sun, X., Dai, X., Lin, C., Shi, Y., Ma, Y. and Li, W., "Environmental Tobacco Smoke (ETS) and Lung Cancer Among Nonsmoking Women in Harbin, China," *Abstracts: Lung Cancer 14 Suppl. 1* (1996), p. S237.

Svendsen, K. H., Kuller, L. H., Martin, M. J., Ockene, J. K., "Effects of Passive Smoking in the Multiple Risk Factor Intervention Trial," *Am J Epid* 126: 783-795, 1987.

Turner, S., Cyr, L., Gross, A.J., "The Measurement of Environmental Tobacco Smoke: Allegations of Scientific Misconduct, *Environment International* 22(2): 263-270, 1996.

Turner, S., Cyr, L., Gross, A.J., "The Measurement of Environmental Tobacco Smoke in 585 Office Environments," *Environ Int* 18: 19-28, 1992. (Provided to Cal/EPA in Philip Morris 1995 Cal/EPA Exposure Comments, Ref. 19.).

U. S. Department of Health and Human Services (U. S. DHHS), "Report of the Surgeon General: The Health Consequences of Involuntary Smoking," DHHS Pub No (PHS) 87-8398, Washington, D. C.

U. S. Environmental Protection Agency (U. S. EPA, 1992), "Respiratory Health Effects of Passive Smoking: Lung Cancer and Other Disorders," Office of Health and Environmental Assessment, Office of Research and Development, U. S. Environmental Protection Agency, 1992.

Wang, S., Hu, Y., Wu, Y., Li, X., Chi, G., Chen, Y., Dai, W., "A Comparative Study of the Risk Factors for Lung Cancer in Guangdong, China," *Lung Cancer 14 Suppl. 1* (1996), pp. 599-5105.

Wang, T-J, Zhou, B-S, Shi J-P, "Lung Cancer in Nonsmoking Chinese Women: A Case-Control Study," *International Symposium on Lifestyle Factors and Human Lung Cancer,* 1994, Guangzhou, People's Republic of China: Paper 6, pp. 1-7, 1995.

Williams, R. B., Barefoot, J. C., Califf, R. M., Haney, T. L., Saunders, W. B., Pryor, D. B., Hlatky, M. A., Sielgler, I.C., Mark, D.B., "Prognostic Importance of Social and Economic Resources among Medically Treated Patients with Angiographically Documented Coronary Artery Disease," *JAMA* 267: 520-4, 1992.

Wu-Williams, A. H., Dai, X. D., Blot, W., Xu, Z. Y., Sun, X. W., Xiao, H. P., Stone, B. J., Yu, S. F., Feng, Y. P., Ershow, A. G., Sun, J., Fraumeni, J. F., Henderson, B. E., "Lung Cancer among Women in Northeast China," *Br J Cancer* 62: 982-987, 1990.

Wu-Williams, A. H., Samet, J. H., "Environmental Tobacco Smoke: Exposure-response Relationships in Epidemiologic Studies," *Risk Analysis* 10: 39-47.

Wynder, E.L., "Response to Science article: Epidemiology faces its limits," *American Journal of Epidemiology* 8: 747-749, 1996.

Yedidia, M. J.,"The Impact of Social Factors on the Content of Care: Treatment of Ischemic Heart Disease at a Public and Voluntary Hospital," *Arch Intern Med* 152: 595-600, 1992.

Young, G. J., Cohen, B. B., "The Process and Outcome of Hospital Care for Medicaid Versus Privately Insured Hospital Patients," *Inquiry* 29: 366-371, 1992.